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Essential Contribution of Monocyte Chemoattractant Protein-1/C-C Chemokine Ligand-2 to Resolution and Repair Processes in Acute Bacterial Pneumonia

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Neutrophils are equipped with various types of machinery for killing bacteria and have crucial roles in the eradication of bacterial infection. However, neutrophils become apoptotic rapidly after killing bacteria, and dying neutrophils release lysosomal enzymes, which eventually cause persistent tissue damage. Thus, engulfment of apoptotic neutrophils is the first step for the successful resolution of inflammation (1–8). Subsequent removal of dying neutrophils is essential to prevent local damage arising from release of injurious contents such as elastase and myeloperoxidase (MPO)2 from dying neutrophils (9). Phagocytosis of dying neutrophils is mainly executed by macrophage lineage cells including alveolar macrophages (AMs) in lung (1, 10, 11).

Resolution of lung inflammation requires the regeneration of bronchial and alveolar epithelium, which are damaged by bacteria and infiltrating neutrophils beforehand. Epithelial regeneration is promoted by several growth factors, particularly hepatocyte growth factor (HGF). HGF was initially detected in the plasma of partially hepatectomized rats as a potent mitogen for mature hepatocytes in primary culture (12–15). Subsequent studies revealed that HGF are mitogenic also for various types of epithelial cells including bronchial and alveolar epithelium. Intratracheal and i.v. administration of recombinant HGF stimulates DNA synthesis of alveolar type II cells in the rat lung after acute lung injury (16, 17). Moreover, exogenously administered HGF attenuated bleomycin-induced lung fibrosis in mice (18). We observed that AMs produce HGF during bacterial pneumonia in mice and that phagocytosis of neutrophils could induce in vitro HGF production by AMs (19).

In most acute bacterial infection, monocytes/macrophages infiltrate into the infection sites, sequentially after neutrophil infiltration. Several lines of evidence indicate that chemokines regulate leukocyte infiltration in concert with adhesion molecules. Among chemokines, monocyte chemoattractant protein (MCP)-1/CC chemokine ligand (CCL)-2 is presumed to have essential roles in monocyte/macrophage recruitment and activation in various diseases (20, 21). Moreover, we previously observed that MCP-1 was protective for systemic lethal infection due to Salmonella typhimurium and Pseudomonas aeruginosa, when administered 6 h before the infection (22). Furthermore, mice deficient in a receptor for MCP-1/CCL2, CCR2, exhibited defects in macrophage recruitment and clearance of bacteria in several organs including liver, spleen, and lung when they were challenged i.v. with Listeria monocytogenes (23). These observations would imply the essential involvement of MCP-1/CCL2 in monocyte/macrophage-mediated protection against bacterial infection. However, it still remains elusive whether MCP-1/CCL2 contributes also to the resolution of inflammation by recruiting and activating AMs.
Here, we examined the roles of MCP-1/CCL2 in acute pneumonia due to *P. aeruginosa*, particularly focusing on the resolution phase. We provided definitive evidence that MCP-1/CCL2 regulated removal of dying neutrophils and HGF production by macrophage, thereby reducing tissue damage.

**Materials and Methods**

**Animals**

Specific pathogen-free, 7-wk-old male Slc:ICR mice were obtained from Charles River Japan (Kanagawa, Japan). The mice were provided with sterile food and water *ad libitum* in an environmentally controlled room. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Nagasaki University School of Medicine.

**Bacteria**

Fisher immunotype 1 *P. aeruginosa* was originally obtained from Dr. M. Pollack (Uniformed Services University of the Health Sciences, Bethesda, MD). After overnight growth on brain-heart infusion agar (Difco, Detroit, MI), the bacteria were harvested in normal saline, resuspended in brain-heart infusion broth (Difco) containing 2% skin milk, and stored at −80°C until use.

**Preparation of mouse MCP-1 and rabbit anti-murine MCP-1 Ab**

Recombinant mouse MCP-1 was prepared as described previously (22). Rabbit anti-mouse MCP-1 Abs were prepared by repeated immunization with recombinant mouse MCP-1 as described previously (24). IgG fractions were purified from immunized or untreated normal rabbit sera by using a protein G-agarose column (Amersham Biosciences, Tokyo, Japan). The resultant IgG can neutralize the lysosomal enzyme release activity of mouse MCP-1 completely at a molar ratio of 100:1 and did not show any cross-reactivities against other members of mouse chemokines including macrophage-inflammatory protein (MIP)-2/CXCL1, MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5 on Western blotting analysis (data not shown).

**Pneumonia model**

To induce nonlethal pneumonia, mice were inoculated intratracheally with 1 × 10⁷ CFU of *P. aeruginosa* as described previously (19), because the LD₅₀ of this strain was 3.0 × 10⁷ CFU in our previous experiments. At the indicated time intervals, bronchoalveolar lavage (BAL) was performed as described below, and lung tissues were obtained for histological examination. After lung tissues were stained with H&E, lung injury was evaluated according to the scoring system described previously (25) by an examiner who had no prior knowledge about the experimental procedures. To determine the cellular source of MCP-1 in the murine lung, immunohistochemical analysis was conducted on lung tissue as described below. In some experiments, anti-mouse MCP-1 or control Abs (250 μg) were administered i.p. to mice twice, before and 24 h after the intratracheal instillation of the bacteria. In another series of experiments, mouse MCP-1 (5 μg) was administered i.p. to the animals 24 h after the intratracheal instillation of the bacteria.

**BAL**

BAL was performed at the indicated time intervals after bacterial challenge as described previously (19). Briefly, trachea was exposed and intubated under deep anesthesia. A 2.5-ml syringe was connected to the tracheal cannula, and the lungs were washed four times with 1.5 ml of Ca²⁺⁻ and Mg²⁺⁻-free PBS at 4°C. A total of 1 ml of BAL fluid was obtained from each mouse. The cell number in BAL fluids were determined with the use of a hemocytometer. Then, the BAL fluids were centrifuged at 150 × g for 10 min for 4°C. Supernatants were sterilized with filters and stored at −80°C until use, whereas cell pellets were resuspended in PBS. Cell monolayer was prepared using Cytospin 2 (Shandon Southern Products, Astmoor, U.K.). Cell morphology was determined on the slides stained with Diff-Quick (Fisher Scientific, Pittsburgh, PA). In addition, we enumerated under an oil immersion microscope apoptotic neutrophils that exhibited nuclear changes characteristic of apoptosis, including condensation of chromatin and simplification of nuclear structure. To observe macrophage engulfment of apoptotic neutrophils, MPO staining was performed as described previously (1, 4, 10). Under these conditions, AMs without any neutrophil engulfment were negative for MPO staining. Then, the macrophages, which were positive for MPO in cytoplasm, were identified as AMs phagocytosing neutrophils. The proportion of AMs phagocytosing neutrophils were determined by counting at least 200 AMs at ×1000.

**ELISA for MCP-1 and HGF**

The concentrations of mouse MCP-1 were determined using ELISA. In brief, a 96-well flat-bottom microtiter plate was coated with 100 μl of rabbit anti-mouse MCP-1 IgG (2 μg/ml) in 0.05 M carbonate buffer (pH 9.6) at 4°C overnight and postcoated with 1% BSA-PBS at 37°C for 2 h. After a washing, 100 μl of either standards or samples were incubated at 4°C for 24 h. Thereafter, the plates were sequentially incubated with biotinylated rabbit anti-mouse MCP-1 IgG (2 μg/ml in 0.5% BSA-PBS) and with streptavidin-conjugated alkaline phosphatase (Life Technologies, Gaithersburg, MD; 1/2000 diluted with 0.5% BSA-PBS) at 37°C for 2 h. After extensive washing, the plate was incubated with 100 μl of p-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO; 1 mg/ml in 1 N diethanolamine, pH 9.8) for 30 min at room temperature, and OD₄₅₀ was read. The concentrations of mouse HGF was determined using the commercially available kit (Institute of Immunology, Tokyo, Japan), according to the manufacturer’s instructions.

**Determination of MPO activities**

MPO activity in BAL supernatants were measured using a freshly prepared solution of 0.34 mM o-dianisidine (3,3'-dimethoxybenzidine; Sigma-
Aldrich) in 0.05 M phosphate-citrate buffer, pH 5.0, containing 0.02% (v/v) of a 30% H$_2$O$_2$ solution as described previously (26). The 40 μl of BAL supernatant or MPO standard (MPO from purulent human sputum; Wako Biochemicals, Osaka, Japan) was added to each well of a flat-bottom 96-well microtiter plate and mixed with 160 μl of the substrate solution; the plate was gently shaken to mix the contents of the well. After 10 min of incubation time at room temperature, the developed coloration was measured with a plate reader at 405 nm.

**Histology**

Lung tissues were fixed with 4% paraformaldehyde in PBS at 4°C for 48 h, dehydrated, and embedded in paraffin. Each section was cut to a thickness of 3 μm and stained with H&E. In some experiments, deparaffinized sections were stained for ssDNA, which arose from single strand breaks in nuclei during programmed cell death using a commercial kit (Dako, Carpinteria, CA), according to the instructions of the manufacturer.

**Immunohistochemical localization of antigenic MCP-1**

Lung tissues were fixed with 4% paraformaldehyde in PBS at 4°C for 48 h, dehydrated, and embedded in paraffin. Each section was cut to a thickness of 3 μm and stained for endogenous MCP-1, with an avidin-biotin-coupling immunoperoxidase technique using a commercial kit (Dako), according to the instructions of the manufacturer. After deparaffinization, the sections were incubated at 4°C overnight with a rabbit IgG against mouse MCP-1 (1/1000) for the primary reaction. After three washes with PBS, the sections were further reacted with biotinylated goat anti-rabbit IgG at room temperature for 2 h. Immune complexes were visualized with 3,3’-diaminobenzidine tetrahydrochloride containing 0.01% hydrogen peroxide. Sections were then counterstained with methyl green for 10 min, dehydrated in ethanol, cleared in xylene, and mounted.

**Preparation of aged neutrophils**

Peritoneal exudate cells were obtained after an i.p. injection of casein according to the method described by Van Epps and Garcia (27). The cells were further purified by centrifugation through mono-poly resolving medium (Dainippon Pharmaceuticals, Osaka, Japan). The resultant cells consisted of ~95% neutrophils as judged by Diff-Quick staining. Freshly isolated neutrophils were incubated in HBSS containing 0.25% BSA (BSA-HBSS) overnight at 37°C in a CO$_2$ incubator. Then, the neutrophils were centrifuged at 150 × g at 4°C, resuspended in DMEM, and used as aging neutrophils. The viability of aging neutrophils was 95–97% by trypan blue exclusion test. The proportion of apoptotic cells in the freshly isolated and aged neutrophils was less than 5.1 ± 0.9 and 21 ± 1.4%, respectively, as determined by detection of annexin V-binding sites with the help of a flow cytometer as described previously (28).

**Coculture of RAW 264.7 cells with aged neutrophils**

A mouse macrophage cell line, RAW 264.7, was cultured in DMEM containing 10% FBS in either a Lab-Tek chamber slide (Nalge Nunc International, Naperville, IL) or a 24-well plate (Nalge Nunc International). On reaching confluence, the cells were washed three times with DMEM without FBS. Then, RAW 264.7 cells were further incubated for 12 h in a
humidified CO₂ incubator in the presence of the indicated concentrations of mouse MCP-1. To assess phagocytic activity, 2 × 10⁶ aged neutrophils were added to RAW 264.7 cells in a Lab-Tek chamber slide and incubated further for 30 min. After noningested neutrophils were removed by extensive washing with ice-cold PBS, MPO staining was performed, and the proportion of RAW 264.7 cells that ingested neutrophils was determined as described previously (1, 4, 10). To determine HGF production, RAW 264.7 cells in a 24-well plate were cultured for 30 min in a humidified CO₂ incubator in the presence of 5 × 10⁶ aged neutrophils. After two washings with ice-cold PBS to remove noningested neutrophils, RAW 264.7 cells

FIGURE 3. Effects of anti-MCP-1 Abs on lung pathology after *P. aeruginosa* pneumonia. A–H, Mice were given either control (A, B, E, and F) or anti-MCP-1 Abs (C, D, G, and H) as described in Materials and Methods. Lung tissues were obtained at 48 h (A–D) or 72 h after *P. aeruginosa* challenge (E–H) and stained with H&E. Representative results from six animals are shown. Original magnification: A, C, E, and G, ×40; B, D, F, and H, ×200. I, Lung injury scores were determined as described in Materials and Methods, and mean ± SE were calculated. *, p < 0.05 when compared with controls.
were further incubated for 24 h in 200 µl of DMEM without FBS. Culture supernatants were collected and filtered for the determination of HGF contents.

Flow cytometric analysis on RAW 264.7 cells

RAW 264.7 cells were cultured in DMEM containing 10% FBS for 12 h in the presence or absence of 10 ng/ml mouse MCP-1 and detached from the dish with 2 mM EDTA in PBS(-) for flow cytometry. The cells (2 × 10^5) in 50 µl of FACS buffer (PBS containing 0.2% BSA, 0.5 mM EGTA, and 10 mM sodium azide) were incubated with an appropriate amount of either one of the following mAbs or isotype-matched control mAb on ice for 30 min: 0.5 µg of FITC-conjugated anti-mouse CD14 rat IgG1 (BD Biosciences, San Jose, CA); 1 µg of FITC-conjugated anti-mouse CD36 mouse IgA (Santa Cruz Biotechnology, Santa Cruz, CA); 0.5 µg of FITC-conjugated anti-mouse CD61 hamster IgG1 (BD PharMingen, San Diego, CA); 10 µg of anti-mouse phosphatidylserine receptor (PS-R) recognizing mouse IgM (Cascade Bioscience, Winchester, MA). After two washings with FACS buffer, the cells incubated with anti-PS-R or its control mAb were further incubated with 1 µg of FITC-conjugated anti-mouse IgG F(ab')2 Ab (American Qualex, San Clemente, CA) on ice for 30 min. Finally, these stained cells were washed twice and analyzed on a FACSscan flow cytometer using CellQuest software (both BD Biosciences, San Jose, CA).

Statistical analysis

Data were expressed as mean ± SE. Statistical analysis was performed by Mann-Whitney’s U test or two-way ANOVA, followed by a multiple comparison according to the Tukey-Kramer procedure. p < 0.05 was accepted as significant.

Results

Leukocyte infiltration in P. aeruginosa pneumonia model

Neutrophils were barely detectable in BAL fluid in untreated mice (Fig. 1A, ○). However, the number of neutrophils in BAL fluid increased rapidly within 24 h after intratracheal instillation of P. aeruginosa, reaching a maximal level at 36 h. The neutrophil number declined thereafter and returned to a basal level by 120 h. A substantial number of macrophages were detected in BAL fluid of untreated mice and intratracheal bacteria instillation induced a significant increase in macrophage number in BAL fluid later than 48 h, increasing gradually thereafter (Fig. 1A, ■). Moreover, macrophage ingesting neutrophils appeared in BAL at 24 h after bacteria instillation (Fig. 1A, □), concomitantly with an increase in neutrophil number in BAL fluid. The number of macrophages ingesting neutrophils increased thereafter, reaching a maximal level at 72 h and decreasing thereafter. We previously observed that macrophages ingested mainly apoptotic neutrophils. Collectively, these results suggest that infiltrating neutrophils became apoptotic.

FIGURE 4. Effects of anti-MCP-1 on parameters in BAL fluid (BALF). Mice were given either control (B, □) or anti-MCP-1 Abs (B, ◊ and ■) as described in Materials and Methods. BAL fluids were obtained at the indicated time intervals. The numbers of neutrophils (A), macrophages (B, □ and ◊), and MPO-positive macrophages in BAL fluid (B, □, control treatment; ■, anti-MCP-1 treatment) were determined as described in Materials and Methods. MPO activities (C) and HGF contents in BAL fluid (D) were determined as described in Materials and Methods. Each group consists of six mice, and mean ± SE were calculated. *, p < 0.05 when compared with controls.
and were ingested by macrophages. Because MCP-1 is a potent chemotactic and activating factor for monocytes/macrophages, we next determined MCP-1 levels in BAL fluid. MCP-1 was barely detected in BAL fluid of untreated animals (Fig. 1B), but MCP-1 levels increased at 24 h after bacteria challenge, preceding the increase in macrophage number in BAL fluid. MCP-1 levels reached a maximal level at 36 h, declining thereafter and returned to a basal level by 120 h.

Immunohistochemical analyses demonstrated the absence of discernible MCP-1 in the lung of untreated animals (Fig. 2, A and B). However, MCP-1 proteins were detected mainly in alveolar type II epithelial cells at 24 h after the infection (Fig. 2, C and D) and in AMs as well as alveolar type II epithelial cells thereafter (Fig. 2, E–H). These observation suggest the intrapulmonary production of MCP-1 after *P. aeruginosa* infection.

**Effects of anti-MCP-1 Abs on *P. aeruginosa*-induced pneumonia**

To assess the roles of endogenously produced MCP-1 in this pneumonia model, we administered anti-MCP-1 Abs to the animals twice, immediately before and 24 h after *P. aeruginosa* challenge.
In lungs of control Ab-treated mice, moderate infiltration into alveoli was observed with moderate hemorrhage and focal exudates at 48 after bacteria instillation (Fig. 3, A and B). At 72 h, neutrophil infiltration was attenuated (Fig. 3, E and F). Anti-MCP-1 Abs further enhanced neutrophil infiltration, hemorrhage, and exudates at 48 and 72 h after bacteria challenge (Fig. 3, C, D, G, and H) and
aggravated lung tissue injury as evidenced by the increased lung injury score (Fig. 3I). Consistent with the effects on neutrophil infiltration, anti-MCP-1 Abs further enhanced the increase in neutrophil number in BAL fluid between 36 and 48 h after bacteria challenge (Fig. 4A). In contrast, the Ab decreased significantly the number of macrophages ingesting neutrophils but not the total macrophage number in BAL fluid (Fig. 4B). Moreover, MPO activities in BAL fluid increased significantly in anti-MCP-1-treated animal compared with control Abs-treated mice, particularly later than 72 h after bacteria challenge (Fig. 4C).

Moreover, the number of neutrophils with condensed chromatin and simplified nuclear structures was significantly higher in BALF from anti-MCP-1 Abs-treated mice than control Ab-treated mice, between 36 and 48 h after bacterial challenge (Fig. 5A). Furthermore, anti-MCP-1 treatment increased the number of neutrophils with ssDNA in lungs, at 36 h after infection (Fig. 5, D and E) as compared with control (Fig. 5, B and C). These results would indicate that anti-MCP-1 Abs reduced the macrophage activity to phagocytose apoptotic neutrophils, a main source of MPO in BAL fluid. Finally, anti-MCP-1 Abs decreased the production of HGF (Fig. 4D), which is presumed to be essentially involved in tissue repair in this pneumonia model.

**Effects of MCP-1 on P. aeruginosa-induced pneumonia**

To assess the roles of exogenously administered MCP-1 in this pneumonia model, we administered MCP-1 to the animals at 24 h after *P. aeruginosa* challenge. The treatment of MCP-1 significantly reduced neutrophil infiltration into alveoli, hemorrhage, and exudates at 48 and 72 h after bacteria instillation (Fig. 6, A–H) and improved lung tissue injury as evidenced by attenuated lung injury score (Fig. 6I). Consistent with the effects on neutrophil infiltration, MCP-1 reduced the increase in neutrophil number in BAL fluid, later than 48 h after bacteria challenge (Fig. 7A). Moreover, MCP-1 increased significantly the number of macrophages ingesting neutrophils as well as total macrophage number in BAL fluid (Fig. 7B). Furthermore, MPO activities in BAL fluid were

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**FIGURE 7.** Effects of MCP-1 on parameters in BAL fluid (BALF). Mice were given either BSA (B, □) or MCP-1 (B, ◊ and ●) as described in Materials and Methods. BAL fluids were obtained at the indicated time intervals. The numbers of neutrophils (A), macrophages (B, □ and ◊), and MPO-positive macrophages in BAL fluid (B: □, control treatment; ●, MCP-1 treatment) were determined as described in Materials and Methods. MPO activities (C) and HGF contents in BAL fluid (D) were determined as described in Materials and Methods. Each group consists of six mice, and mean ± SE were calculated. *, p < 0.05 when compared with controls.
Effects of MCP-1 on the expression of apoptosis recognition receptors on a macrophage cell line, RAW 264.7

We finally evaluated the effects of MCP-1 on the expression of receptors (PS-R; CD14, CD36, and CD61), which are presumed to be involved in phagocytosis of apoptotic cells, by using RAW 264.7 cells as a representative of macrophages. MCP-1 augmented the expression of PS-R, but not other receptors (Fig. 9). These data suggest that MCP-1 may augment the macrophage capacity to phagocytose apoptotic cells by increasing expression of the PS-R.

Discussion

Neutrophil infiltration is the first step to eradication of bacterial infection. However, neutrophils become apoptotic and release injurious substances as soon as they phagocytose bacteria. Persistent presence of dying neutrophils cause tissue damage and eventually organ dysfunction. Thus, removal of apoptotic neutrophils is required for reducing organ dysfunction and promoting tissue repair (29). We previously observed that intratracheal instillation of P. aeruginosa caused a rapid neutrophil infiltration into airspace, followed subsequently by macrophage accumulation. Moreover, accumulated macrophages phagocytosed neutrophils and produced HGF, a potent growth factor for pulmonary epithelial cells. However, it remains elusive how macrophages are recruited and activated in this process.

Accumulating evidence indicates that MCP-1/CCL2 has crucial roles in monocyte/macrophage recruitment and activation in various types of diseases. Hence, we examined the roles of MCP-1/CCL2 in P. aeruginosa-induced pneumonia, particularly focusing on the removal of neutrophils and HGF production by AMs. The administration of anti-MCP-1 Abs enhanced neutrophil infiltration without any effects on macrophage accumulation. However, anti-MCP-1/CCL2 Abs reduced significantly the number of macrophages ingesting neutrophils. Moreover, the number of apoptotic neutrophils in BAL fluid and alveolar space in the lung were increased in the animals treated with anti-MCP-1 Abs. In contrast, MCP-1 treatment reduced neutrophil infiltration and macrophage accumulation and increased the numbers of macrophages ingesting neutrophils. Collectively, these observations suggest that MCP-1/CCL2 was involved mainly in ingestion of apoptotic neutrophils by macrophages but not macrophage recruitment.

Neutrophils rapidly undergo apoptosis upon bacterial phagocytosis. If apoptotic neutrophils are not removed by macrophages, they become necrotic and release a number of potentially injurious substances including MPO (9), which subsequently can cause tissue injury through direct cell lysis (30). Anti-MCP-1 treatment sustained elevated MPO levels in BAL fluids for 24 h after infection and aggravated tissue injury, whereas MCP-1 treatment reduced MPO levels as well as tissue damage. These observations suggest that MCP-1 reduces tissue injury by enhancing removal of apoptotic neutrophils by macrophage, which thereby prevents the release of injurious substances such as MPO from neutrophils.

We previously observed that MCP-1 could enhance macrophage activities to phagocytose and kill P. aeruginosa in vitro (22). However, in this pneumonia model, no significant changes in bacteria load in lungs were observed at 24 h after the infection by the administration of either anti-MCP-1 Ab or MCP-1 (our unpublished data). Consistent with these observations, Knapp et al. (31) demonstrated that MCP-1 reduces tissue injury by enhancing removal of apoptotic neutrophils by macrophage, which thereby prevents the release of injurious substances such as MPO from neutrophils.
demonstrated that selective depletion of AMs decreased clearance of apoptotic neutrophils without any changes in intrapulmonary bacteria load in experimental pneumococcal pneumonia. Moreover, uncleared apoptotic neutrophils became necrotic and prevented the resolution of inflammation, thereby causing a higher mortality than control mice. Thus, it is tempting to speculate that MCP-1-mediated macrophage recruitment and activation was mainly involved in the clearance of apoptotic neutrophils and subsequent resolution of acute inflammation.

Anti-MCP-1 did not inhibit macrophage accumulation at all. Moreover, anti-MCP-1 treatment exhibited only partial inhibitory effects on clearance of apoptotic neutrophils. In pneumococcal pneumonia in mice, Fillion et al. (32) described the combined administration of Abs against MCP-1, MIP-1α, or RANTES, but not a single type of Abs reduced monocyte/macrophage recruitment significantly. Thus, it is presumed that several additional chemokines regulated macrophage accumulation in concert with MCP-1. However, it cannot be excluded that the dose of the Abs used in these experiments was not sufficient to show complete inhibitory effects.

Evidence is accumulating that HGF stimulates proliferation of bronchial and alveolar type II epithelial cells through DNA synthesis in vivo as well as in vitro (16, 17, 33, 34). Moreover, Yamada et al. (25) demonstrated that the neutralization of HGF strongly inhibited DNA synthesis of alveolar epithelial cells in the ischemic-reperfused lung injury. Furthermore, several lines of evidence would indicate that HGF could prevent apoptosis (34, 35) and induce survival for pheochromocytoma PC12 cells in vitro (36). Because HGF levels in BAL fluid were correlated well with the degree of tissue damage even in our model, HGF may be involved in repair process of damaged lung tissues by promotion of proliferation and prevention of apoptosis.

Low et al. (37) observed that MCP-1-deficient mice exhibited delayed re-epithelialization, angiogenesis, and collagen synthesis in skin wound, suggesting potential roles of MCP-1 in tissue repair. Consistent with their observation, in our pneumonia model, anti-MCP-1 Abs and MCP-1 aggravated and reduced lung injury, respectively. Because MCP-1 could enhance HGF production by macrophages, particularly those that phagocytosed neutrophils, MCP-1 can promote tissue repair by inducing the production of a potent pulmotropic factor, HGF.

Although MCP-1 augmented a macrophage cell line, RAW 264.7, to ingest apoptotic neutrophils, it activates RAW 264.7 cells to produce HGF only in the presence of apoptotic neutrophils. A
variety of receptors are involved in the removal of apoptotic cells, such as lectin-like receptors (38), the vitronectin receptor, CD36 (11), PS-R (39, 40), CD14 (41), and scavenger receptors (42). Our present flow cytometric analysis demonstrated that MCP-1 augmented the macrophage expression of PS receptor but not other receptors. Because PS-R has a dominant role in the recognition of apoptotic cells by interacting with PS exposed selectively on apoptotic cells, MCP-1 may augment the macrophage activity to phagocytose apoptotic cells by enhancing the expression of PS-R.

Bacteria and their products can activate a transcription factor, NF-κB, through interaction with Toll-like receptors and eventually cause the production of chemokines including MCP-1. However, mouse macrophage exhibited reduced production of most chemokines except MCP-1 when they ingested apoptotic cells (43). MCP-1 induced the production of anti-inflammatory cytokine, IL-10, in endotoxin shock (44). Thus, MCP-1 may be responsible for IL-10 production by monocytes that were cocultured with endotoxin and apoptotic cells (45). Moreover, we previously observed that IL-10 augmented MCP-1 production by AMs (46). Furthermore, we observed that alveolar type II epithelial cells produced MCP-1 in this model, consistent with the previous in vitro data (47). These observations suggest the presence of positive feedback mechanisms between MCP-1 and IL-10, which may dampen inflammatory reaction in a coordinate manner. However, either anti-MCP-1 Ab or MCP-1 have marginal effects on BAL fluid IL-10 levels after bacterial infection from 24 h until 120 h after the infection. Thus, IL-10 has few, if any, roles in resolution of this acute bacterial infection model.

We previously observed that MCP-1 administration at the early phase was protective against several bacterial infection by enhancing killing of bacteria by macrophages (22). Here, we further provide evidence that MCP-1 administration at the later phase of infection could promote the tissue repair process in bacterial pneumonia by induction of removal of apoptotic neutrophils and HGF production by AMs. Therefore, MCP-1 may be an additive measure for patients with severe bacterial pneumonia, to prevent infection-induced tissue destruction.

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References


