Acid and particulate-induced aspiration lung injury in mice: importance of MCP-1

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chemokines, keratinocyte-derived cytokine (KC) and macrophage inflammatory protein-2 (MIP-2), that function as dual homologs of IL-8. KC is also the mouse homolog of cytokine-induced neutrophil chemoattractant-1 (CINC-1) in rats. These cytokines and chemokines were chosen for study because they have previously been found to play a role in the pathogenesis of lung injury in rats, predict the severity of pulmonary dysfunction, and/or be helpful in the discrimination of different forms of gastric aspiration in rats (6, 16, 33). Survival at the 48-hour period following gastric aspiration is also assessed as an outcome variable, and lung tissue from WT and MCP-1(-/-) mice is examined to elucidate differences in histological patterns of pulmonary injury.

MATERIALS AND METHODS

Aspiration Lung Injury

All animal experiments were approved by the Institutional Committee for Animal Care and Use at the University at Buffalo-State University of New York. Before study, specific pathogen-free adult C57BL/6 (WT) mice and MCP-1(-/-) mice on a same C57BL/6 background were housed in high efficiency particulate air filter-topped cages in a sterile laminar flow environment. MCP-1(-/-) mice were a gift from Dr. B. J. Rollins (Department of Adult Oncology, Dana-Farber Cancer Institute) (20). Mice were anesthetized with 2% halothane in oxygen at a rate of 5 l/min. After induction of anesthesia, the trachea was exposed with a 1-cm longitudinal incision, and a 22-gauge needle was introduced. Animals received an intratracheal aspirate at a uniform volume of 3.6 ml/kg containing either normal saline, pH 5.3 (NS, vehicle control); NS+HCl, pH 1.25 (ACID); 40 mg/ml gastric particles in normal saline, pH 5.3 (SNAP); or 40 mg/ml gastric particles in normal saline plus HCl, pH 1.25 (CASP). The volumetric dose of 3.6 ml/kg was chosen based on preliminary experiments showing that WT mice receiving this amount of HCl (pH 1.25) had minimal mortality and an increase in albumin permeability index similar to our prior studies of acid aspiration in rats (6, 33). After instillation of ACID, SNAP, or CASP, the intratracheal catheter was removed, and the tracheal incision was repaired with a 6-0 Ethilon suture. The skin incision was closed with surgical staples, and the mice were placed in room air until being killed at 5, 24, or 48 hours for lung injury assessments. To further define differences between MCPP(-/-) and WT mice, additional studies were also done with CASP aspirates containing particulate concentrations of 5, 10, or 20 mg/ml. Gastric particles in all cases were prepared from the stomach contents of C57BL/6 WT mice, which were washed several times in NS, filtered through gauze, and sterilized by autoclaving (final particle sizes were <30 μm) (18).

BAL

At 5, 24, or 48 hour postaspiration, mice were reanesthetized with 2% halothane, and a midline abdominal/thoracic incision was made to allow exsanguination via the inferior vena cava. The inferior vena cava was then clamped just below the diaphragm, and the thoracic and pulmonary vasculature flushed with 5 ml of Hanks’ balanced salt solution (HBSS) containing Ca2+ and Mg2+ (Life Technologies, Grand Island, NY) introduced through the right ventricle. The neck incision was reopened, and a 22-gauge cannula was secured in the trachea with a suture. BAL was performed with five aliquots (1 ml each) of HBSS with Ca2+ and Mg2+, as described previously (16, 22). Recovered lavage fluid was pooled for cell and cytokine analysis. The percent recovery of instilled lavage fluid was 82 ± 1.7% (means ± SE).

Cell Analyses

Cells were immediately pelleted from BAL by centrifugation at 1,500 g for 3 min at 4°C (GS-6 centrifuge with GH-3.5 rotor; Beckman Coulter, Fullerton, CA), and the cell-free supernatant was stored at −80°C for later analyses of albumin and cytokine content. The cell pellet was resuspended in 2 ml of phosphate-buffered saline (PBS, pH 7.2) containing 0.1% sodium azide and carefully layered on top of 3 ml of 2% (wt/vol) bovine serum albumin (BSA) in PBS in a 12 × 75-mm polystyrene tube. The cell suspension was centrifuged for 10 min at 150 g at 4°C, the supernatant was aspirated, and the pellet resuspended in 1 ml of ice-cold 0.1% sodium azide in PBS. A 200-μl aliquot was diluted 1:50 in Isoton II solution (Beckman Coulter), and the leukocyte concentration was determined with a Multisizer 3 Coulter Counter (Beckman Coulter). A cytospin was prepared with 5 × 10⁴ leukocytes using a Cytospin 3 cytocentrifuge (Shandon, Pittsburgh, PA) and stained with Diff-Quik reagents (Baxter, Miami, FL), and a differential leukocyte count was determined by light microscopy.

Cytokine Analyses

Cytokine concentrations in cell-free BAL were determined with ELISA kits from R&D Systems (TNF-α, IL-1β, MIP-2, IL-6, IL-10, and KC; Minneapolis, MN) and Pharmingen (MCP-1; San Diego, CA) according to manufacturer’s instructions.

Albumin Leakage

Albumin leakage was assessed by ELISA using a polyclonal rabbit anti-mouse albumin antibody (provided by Dr. Daniel Remick, University of Michigan, Ann Arbor, MI), horseradish peroxidase-labeled goat anti-rabbit IgG (Pharmingen, San Diego, CA), and mouse albumin as a standard (Sigma, St. Louis, MO) (16, 22).

Histopathology

Lung tissue was harvested at 24 hour postaspiration in WT and MCP-1(-/-) mice and was immediately fixed in 10% phosphate-buffered formalin. Fixed tissue samples were processed in paraffin blocks, and fine sections (4 μm in thickness) were cut with a microtome and stained with hematoxylin and eosin (H&E) for examination by light microscopy. Assessments of histopathology were performed in blinded fashion by an experienced pathologist (J. A. Woytash).

Statistical Methods

All data are expressed as means ± SE. A 2 × 2 factorial design was used to compare lung injury following aspiration of CASP, SNAP, ACID, or NS at 5, 24, or 48 hours, controlling for mouse type with respect to two cellular and seven cytokine biomarkers of lung injury. A secondary analysis compared animal mortality as a function of CASP injury (5–40 mg particles/ml). Data were analyzed at each time point using a log-normal model with two factors corresponding to injury type (ACID or SNAP were coded as indicator variables, with the ACID-SNAP interaction term corresponding to CASP). An additional factor for mouse type was also included in the model [WT = 0, MCP(-/-) = 1]. To properly account for observations below the level of detection, we treated these values as left-censored at the detection limit. The model was fit via standard maximum-likelihood methods, with the error rate over multiple factors controlled with a Bonferroni corrected level of α = 0.05/3 = 0.0167. Additionally, intergroup comparisons at specific time points were made using one-way ANOVA with a Bonferroni corrected level of α = 0.05/6 = 0.0083. Mortality was analyzed using a standard Cox proportional-hazards model and summarized with estimates of relative risk as a function of CASP particulate level. All statistical tests were done using SAS/STAT version 9.0 (SAS Institute, Cary, NC).
RESULTS

Aspiration Lung Injury Model From ACID, SNAP, or CASP in WT Mice

Initial studies developed a C57BL/6 WT mouse model of inflammatory lung injury for three forms of aspiration known to generate distinct responses in rats: CASP (40 mg particles/ml), SNAP (40 mg particles/ml), and ACID (6, 16, 33).

Albumin leakage. Albumin concentration in BAL from WT mice was increased in all three forms of aspiration compared with NS controls at 5 h and remained elevated over the full 48-h period of study (Fig. 1). WT mice given CASP had a synergistic increase in albumin leakage at 5 h compared with WT mice given ACID or SNAP alone (1,261 ± 204 μg/ml for CASP compared with 123 ± 13 μg/ml and 328 ± 48 μg/ml for ACID and SNAP, respectively, P < 0.0083) (Fig. 1). CASP-induced lung injury in WT mice also persisted at a more severe level of albumin leakage for a longer duration. At 48 h postaspiration, there was still considerable albumin in the air spaces of WT mice given CASP (755 ± 107 μg/ml for CASP compared with 139 ± 20 μg/ml for ACID and 295 ± 40 μg/ml for SNAP, P < 0.0083) (Fig. 1). These results showing synergistic increases in albumin levels in BAL from WT mice given CASP compared with ACID or SNAP are conceptually similar to findings reported in rats (6, 16, 33).

Leukocyte infiltration. The numbers of neutrophils and macrophages in BAL from WT mice given ACID, SNAP, or CASP are shown in Fig. 2. Neutrophil numbers in BAL from WT mice with SNAP or CASP injury were significantly elevated at the earliest time studied (from 2.7 ± 1.8 × 10^3 cells at 0 h to 2.7 ± 0.4 × 10^6 cells for SNAP and 2.0 ± 0.4 × 10^6 cells for CASP at 5 h, P < 0.0083, Fig. 2A). WT mice receiving ACID had a smaller increase in neutrophils in BAL at 5 h compared with either SNAP or CASP (1.8 ± 0.4 × 10^5 cells, P < 0.0083). A similar pattern of increased neutrophil numbers in BAL in the order of CASP > SNAP > ACID was also present at 24 and 48 h postaspiration (Fig. 2A). In contrast, macrophage numbers in BAL were reduced at 5 h in WT mice given SNAP or CASP (5.2 ± 1.0 × 10^5 cells for SNAP and 6.4 ± 1.1 × 10^5 cells for CASP compared with 1.7 ± 0.3 × 10^6 cells at baseline, P < 0.0083) and returned to control levels at 24
and 48 h (Fig. 2B). These macrophage responses in WT mice differ from those in rats, which have been found to exhibit early increases in macrophage numbers in BAL following CASP or SNAP aspiration and have minimal changes in macrophage numbers after ACID (16).

**Inflammatory mediators.** Levels of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 in BAL had distinct patterns of elevation in WT mice following the instillation of ACID, SNAP, or CASP (Fig. 3). Concentrations of TNF-α in BAL were elevated at 5 h in WT mice receiving SNAP compared with other forms of aspiration ($P < 0.0083$, Fig. 3A). By 24 h, levels of TNF-α in BAL approached baseline in all forms of aspiration injury and remained low at 48 h. IL-1β was increased in BAL from WT mice at 5 h following CASP or SNAP ($P < 0.0083$ compared with saline controls) (Fig. 3B) and remained elevated at 24 and 48 h for animals receiving CASP. Levels of IL-6 in BAL were significantly increased at 5 h for WT mice instilled with SNAP or CASP compared with NS controls ($P < 0.0083$, Fig. 3C). Levels of IL-6 in BAL were also increased over NS controls for WT mice given ACID, although the magnitude of the increase was less than for CASP or SNAP. Levels of IL-6 in BAL from WT mice given CASP remained significantly elevated at 24 and 48 h postaspiration compared with NS controls (Fig. 3C). Levels of IL-6 in BAL demonstrated the strongest correlation with lavaged albumin concentrations at 24 and 48 h in the murine model ($r = 0.8180$, $P < 0.001$).

Levels of the CXC neutrophil chemoattractants KC and MIP-2 and the monotypic CC chemokine MCP-1 were elevated most prominently in WT mice with CASP or SNAP aspiration (Fig. 4). At 5 h, KC concentrations in BAL were $1,180 \pm 260$ pg/ml for CASP and $531 \pm 46$ pg/ml for SNAP ($P < 0.0083$ compared with saline controls, Fig. 4A). Levels of MIP-2 in BAL were also significantly elevated at 5 h for WT mice given CASP or SNAP ($1,580 \pm 150$ pg/ml and $1,530 \pm 150$ pg/ml, respectively; $P < 0.0083$ compared with saline controls, Fig. 4B). Levels of KC and MIP-2 remained elevated at 24 h for WT mice given CASP or SNAP ($P < 0.0083$ compared with control; Fig. 4, A and B), with KC returning to baseline at 48 h. Levels of MCP-1 were greatly elevated at 24 h in BAL from WT mice given CASP ($408 \pm 38$ pg/ml, $P < 0.0083$), and this chemokine was also increased to a lesser extent in WT mice given SNAP ($223 \pm 28$ pg/ml, Fig. 4C). Levels of MCP-1 in BAL were increased further at 48 h in WT mice given CASP ($774 \pm 173$ pg/ml, $P < 0.0083$) and remained elevated to a lesser extent at this time in WT mice given SNAP or ACID (Fig. 4C). Measurements of IL-10 in BAL from mice with aspiration lung injury were also performed, but levels were below or near the lower limit of assay detection in WT mice (data not shown). Low levels of IL-10 in
WT mice with CASP are in contrast with results in rats, which exhibit a significant IL-10 response to aspiration injury (16).

Results Comparing Mortality and the Severity of Lung Injury From CASP Aspiration in WT Mice vs. MCP-1(−/−) Mice

A second set of experiments investigated the specific functional importance of MCP-1 in the substantial aspiration lung injury caused by CASP. This was done by comparing survival and the severity of inflammatory lung injury in WT mice and MCP-1(−/−) mice given CASP aspirates containing a range of doses of gastric particulates (5–40 mg particles/ml).

Mortality in MCP-1(−/−) and WT mice given CASP. MCP-1(−/−) mice had substantially greater mortality than WT mice following intratracheal instillation of CASP with the standard particulate dose of 40 mg/ml used in the previous section. Only 32% of MCP-1(−/−) mice survived this level of CASP at 24 h, and none survived at 48 h (Fig. 5). WT mice had corresponding survival rates of 80% at 24 h and 72% at 48 h following the instillation of CASP (40 mg/ml). Large numbers of mice were studied to validate these survival data, particularly at 24 h when survival differences first became pronounced [n = 28 and 31, respectively, for WT and MCP-1(−/−) mice at 24 h in Fig. 5]. Additional studies assessed mortality in MCP-1(−/−) and WT mice after the instillation of CASP containing lower levels of particulates (5, 10, 20 mg/ml in the same instillation volume of 3.6 ml/kg). There was no mortality over 48 h in WT mice given these lower particulate levels of CASP, but MCP-1(−/−) mice still had substantial mortality at CASP particulate concentrations of 10 or 20 mg/ml. MCP-1(−/−) mice given CASP with 5 mg/ml particulates had survival equivalent to WT mice given CASP with 40 mg/ml particulates. According to a Cox proportional-hazard model, MCP-1(−/−) mice that received 40 mg/ml CASP were 11 times more likely to die than those given 5 mg/ml CASP. MCP-1(−/−) mice given 10 or 20 mg/ml CASP were 3.9 and 4.6 times more likely to die, respectively, compared with those given 5 mg/ml CASP.

Albumin leakage in MCP-1(−/−) and WT mice given CASP. There were no significant differences between surviving WT and MCP-1(−/−) mice in albumin concentrations in BAL at 5 or 24 h following the instillation of CASP with 40 mg/ml particulates (Fig. 6A). Comparisons between MCP-1(−/−) and WT mice with CASP at this particulate level were limited to 5 and 24 h postaspiration, since all the MCP-1(−/−) mice died before 48 h. Albumin levels in BAL at 5 h were greater for MCP-1(−/−) mice compared with WT mice following the instillation of CASP with a low 5 mg/ml particulate level (P < 0.05, Fig. 6B). However, no difference could be detected at 24 and 48 h between albumin levels in BAL from MCP-1(−/−) mice and WT mice given 5 mg/ml CASP.
Leukocyte numbers in MCP-1(−/−) and WT mice given CASP. Surviving MCP-1(−/−) and WT mice had similar patterns of elevation of neutrophils (PMNs) and macrophages in BAL for CASP containing a fixed level of particulates (Fig. 7). Numbers of PMNs and macrophages in BAL from both types of mice were greater for 40 mg/ml CASP compared with 5 mg/ml CASP, except for WT mice at 48 h. However, correlations between the particulate level of CASP and leukocyte numbers in BAL were largely independent of mouse genotype. MCP-1(−/−) mice had substantial numbers of both PMNs and macrophages in BAL, indicating that recruitment of leukocytic cells, including monocytes, still occurred in these knockout mice. Statistical analysis of combined data for both mouse types [MCP-1(−/−) and WT] indicated that the total number of PMNs plus macrophages in BAL correlated strongly with lung injury severity based on albumin concentrations at 24 h ($r^2 = 0.40$, $P < 0.0001$). Numbers of PMNs alone in BAL correlated best with albumin concentrations at 48 h ($r^2 = 0.41$, $P < 0.0001$).

Cytokine/chemokine levels in MCP-1(−/−) and WT mice with CASP injury. Lung injury from CASP in both MCP-1(−/−) and WT mice was associated with increases in the levels of multiple inflammatory mediators in BAL. Mediator levels in BAL were generally greatest for mice receiving CASP with a greater particle load (40 mg/ml vs. 5 mg/ml). At a fixed particulate level of CASP, concentrations of TNF-α, IL-1β, and IL-10 in BAL did not differ substantially between MCP-1(−/−) and WT mice. However, concentrations of KC and IL-6 in BAL did exhibit significant differences between the two types of mice depending on time and CASP particle dose (Fig. 8). Differences in KC in BAL were found between MCP-1(−/−) and WT mice given 40 mg/ml CASP (Fig. 8A). Levels of KC in BAL were significantly increased in MCP-1(−/−) mice at 24 h after aspiration of 40 mg/ml CASP but peaked earlier and then decreased at 24 h in WT mice given the

**Fig. 5.** Cumulative survival in MCP-1 knockout [MCP-1 (−/−)] and WT mice given CASP. Cumulative survival is shown for MCP-1(−/−) mice and WT mice at 5, 24, and 48 h following intratracheal instillation of CASP (40 mg particles/ml). MCP-1(−/−) mice had significantly decreased survival compared with WT mice for CASP aspirates containing this standard particulate dose used in Figs. 1–4. Survival differences were also present for MCP-1(−/−) mice and WT mice given CASP with lower particulate doses of 10 and 20 mg/ml as described in the text. Numbers of mice studied were $n = 9$ for WT and MCP-1(−/−) at 5 h; $n = 31$ for WT and 28 for MCP-1(−/−) at 24 h; and $n = 11$ for WT and 8 for MCP-1(−/−) at 48 h.

**Fig. 6.** Levels of albumin in BAL from MCP-1(−/−) and WT mice given CASP containing particle loads of 40 and 5 mg/ml. **A**: CASP, 40 mg particles/ml. **B**: CASP, 5 mg particles/ml. Levels of albumin ($\mu g$/ml) in BAL from surviving MCP-1(−/−) mice and WT mice given CASP (40 mg/ml) were not statistically different (comparisons are limited to <24 h since no MCP-1(−/−) mice survived at 48 h). Albumin levels in BAL from MCP-1(−/−) mice given CASP (5 mg/ml) were elevated at 5 h compared with corresponding WT mice. *$P < 0.05$. Data are means ± SE, $n = 9–14$. fig.6a.png

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same aspirate. For injury with 5 mg/ml CASP, MCP-1(−/−) mice had substantially higher levels of IL-6 in BAL at 5, 24, and 48 h compared with WT mice (Fig. 8B). Also, consistent with the lack of a functional gene, MCP-1(−/−) mice had undetectable levels of this CC chemokine at all times studied, whereas WT mice had significant levels of MCP-1 following CASP injury with high or low particle loads (Fig. 8C). In terms of statistical correlations combining data for both mouse types [MCP-1(−/−) and WT mice], levels of IL-6 in BAL were the best predictor of lung injury severity based on albumin leakage at 5 h after CASP ($r^2 = 0.48, P < 0.0001$).

**Histological assessments of CASP aspiration injury in MCP-1(−/−) and WT mice.** Lung tissue sections stained with H&E were examined to gain further insight about the increased mortality in MCP-1(−/−) mice with CASP aspiration injury (Fig. 9). Tissue sections were obtained at 24 h postaspiration of 40 mg/ml CASP, a time when substantial survival differences between MCP-1(−/−) and WT mice existed (Fig. 5). Gross observation at the time when lung tissue samples were obtained did not identify any intra-abdominal or thoracic pathology in either MCP-1(−/−) or WT mice. However, microscopic examination of H&E-stained sections revealed substantial differences in the pattern of CASP tissue injury between the two types of mice. WT mice had prominent granuloma formation in lung tissue, with circumscribed areas of organizing (consolidated) injury (Fig. 9A). In contrast, MCP-1(−/−) mice had little or no evidence of granuloma formation, with a pattern of severe diffuse tissue injury consistent with acute bronchopneumonia (Fig. 9B), suggesting that this CC chemokine played an important role in compartmentalizing the inflammatory response thereby protecting the uninjured lung.

**DISCUSSION**

The results of this study show that aspiration of CASP results in a more severe inflammatory lung injury in C57BL/6 WT mice compared with aspiration of either acid or particulates alone. WT mice receiving CASP at a standard 40 mg/ml particulate level had higher albumin concentrations, greater numbers of PMN neutrophils, and increased levels of inflammatory mediators in BAL compared with WT mice given ACID or SNAP alone (Figs. 1–4). These results verify the hypothesis that WT mice respond to two-hit gastric aspiration with increased lung injury in a conceptually similar fashion to that demonstrated previously in rats (6, 16, 33). In addition, experiments studying mice deficient in the gene for MCP-1 supported the hypothesis that this mononuclear chemokine is important in protecting against progressive lung injury from CASP aspiration. MCP-1(−/−) mice had severe inflammatory lung injury following the tracheal instillation of CASP, and at a fixed gastric particulate dose exhibited remarkably higher mortality compared with WT mice (Fig. 5). Increases in mortality were most apparent in MCP-1(−/−) mice given 40 mg/ml CASP, but these mice also had decreased survival compared with WT mice following the aspiration of CASP containing lower particle levels of 10 or 20 mg/ml CASP. MCP-1(−/−) mice given 5 mg/ml CASP had survival similar to WT mice given 40 mg/ml CASP.

Mortality differences between MCP-1(−/−) and WT mice given 40 mg/ml CASP did not correlate strongly with differences in lung injury severity based on levels of albumin or leukocytes in BAL from survivors (Figs. 6 and 7). Surviving MCP-1(−/−) mice did have increased albumin levels in BAL at 5, 24, and 48 h compared with WT mice (Fig. 8B). Also, consistent with the lack of a functional gene, MCP-1(−/−) mice had undetectable levels of this CC chemokine at all times studied, whereas WT mice had significant levels of MCP-1 following CASP injury with high or low particle loads (Fig. 8C). In terms of statistical correlations combining data for both mouse types [MCP-1(−/−) and WT mice], levels of IL-6 in BAL were the best predictor of lung injury severity based on albumin leakage at 5 h after CASP ($r^2 = 0.48, P < 0.0001$).
and WT mice in terms of albumin, cells, and inflammatory mediators in BAL following 40 mg/ml CASP were affected by the higher mortality of these animals compared with WT mice at both 24 and 48 h. MCP-1 mice that failed to survive, and hence were not evaluated in terms of lung injury and inflammatory parameters, may have had significantly more pronounced (or differing) responses than those reported here.

Although the present study focused on injury to the lungs, it is possible that extrapulmonary pathology contributed to the increased mortality observed in MCP-1 mice vs. WT mice given CASP. Our studies did not investigate pathology in other organ systems and also did not quantitate levels of circulating inflammatory mediators (systemic inflammatory responses as opposed to local inflammatory responses as assessed in lung lavage). Because MCP-1 has diverse biological effects as detailed below, the absence of this chemokine in genetically deficient mice may have led to significant differences in pathology not only in the lungs (e.g., Fig. 9) but in other organ systems as well. This possibility should be investigated in more detail in future studies on the roles of MCP-1 in inflammatory responses to gastric aspiration.

Several known activities of MCP-1 may be related to our finding that this mediator is important in protecting against mortality at 24 and 48 h in mice with CASP aspiration (Fig. 5). MCP-1 is a 76-amino acid peptide belonging to the CC family of chemokines (37). It has been shown to have chemoattractant properties not only for monocytes, but also for basophils and mast cells (5, 37). MCP-1 is produced by immune and nonimmune cells in response to a variety of stimuli including TNF-α, IL-1β, IL-4, viruses, and endotoxin (5). MCP-1 has significant activity in the acute inflammatory response (5, 14, 34, 37). In addition to monocyte chemotactic activity, MCP-1 has also been shown to induce a respiratory burst, upregulate the expression of β2-integrin, and trigger cytokine secretion by peripheral blood monocytes (14). Working with LPS-induced endotoxemia in mice, Zisman et al. (37) showed that neutralization of MCP-1 led to significant increases in plasma and lung levels of TNF-α and IL-12. In the present study, surviving MCP-1 mice did not have significantly elevated levels of TNF-α compared with WT mice given 5 or 40 mg/ml CASP.

Flory et al. (7) have demonstrated that MCP-1 plays a role in the formation of angiocentric granulomas following infusion of yeast cell wall glucans in rats. This is consistent with our results that a major histological difference in lung tissue at 24 h after CASP aspiration was the presence of organizing injury with granuloma formation in WT mice, whereas MCP-1 mice with increased mortality had severe diffuse pneumonia without evidence of granuloma formation (Fig. 9). Granuloma formation reflects the host reaction to contain or compartmentalize the immune/inflammatory response to a noninfectious insult or an infectious agent as demonstrated, for example, in mycobacterial infections (8, 32). The ability to generate gran-
Listeria monocytogenes intracellular pathogens including mice overexpressing MCP-1 have an increased susceptibility to overexpression of MCP-1 is not beneficial, since transgenic production by alveolar macrophages (1, 21). At the same time, dying neutrophils and augmenting hepatocyte growth factor and acute bacterial pneumonia by enhancing the removal of a role in the resolution and repair of airway epithelial injury monas aeruginosa pate in the innate immune response required to clear mechanism or process is involved in the survival differences of MCP-1 has been shown to be involved in cell-mediated im-

ative processes other than granuloma formation. For example, MCP-1 has been shown to be involved in cell-mediated immunity against Cryptococcus neoformans (13) and to partici-
pate in the innate immune response required to clear Pseudomonas aeruginosa and Salmonella typhimurium following intraperitoneal injection in mice (27). Recent studies have indicated that MCP-1, along with CC chemokine ligand-2, has a role in the resolution and repair of airway epithelial injury and acute bacterial pneumonia by enhancing the removal of dying neutrophils and augmenting hepatocyte growth factor production by alveolar macrophages (1, 21). At the same time, overexpression of MCP-1 is not beneficial, since transgenic mice overexpressing MCP-1 have an increased susceptibility to intracellular pathogens including Mycobacterium tuberculosis and Listeria monocytogenes (31). The diverse biological effects of MCP-1 make it very likely that more than just one mechanism or process is involved in the survival differences found here between MCP-1(−/−) and WT mice with CASP aspiration.

We have previously developed and investigated a rat model for acute aspiration pneumonitis associated with the tracheal instillation of ACID, SNAP, and CASP (6, 15, 16, 18, 26, 33). Although some differences in specific inflammatory mediator responses were found here in WT mice relative to our earlier results in rats, a similar pattern of increased inflammation and lung injury severity associated with CASP compared with ACID or SNAP alone was clearly present in both species. The synergism of injury severity in CASP aspiration appears to involve potentiation of an early chemical injury by ACID by continued irritation and inflammation from particulate matter in SNAP (18). ACID-induced lung injury in rats has been described as including a chemical response that peaks at 1 h (18). The present murine experiments did not investigate this very early time point. However, at 5 h postaspiration, ACID did not cause as great an acute neutrophilic infiltration in WT mice (Fig. 2A) compared with the aspiration of SNAP or CASP. Levels of inflammatory mediators in BAL were also very low in WT mice receiving ACID compared with either SNAP or CASP at 5–48 h (Figs. 3 and 4).

The present experiments indicated substantial similarities between inflammatory mediator responses to different forms of aspiration in WT mice relative to prior results in rats for the production of antigenic levels of TNF-α, IL-1β, MIP-2, and CINC-1 (CXC chemokine homolog for murine KC) in BAL (6, 16, 33). The most notable difference observed between inflammatory mediator responses for the two species involved IL-10 and IL-6. In the rat, levels of IL-10 in BAL have been reported to be the best single predictor of the severity of aspiration lung injury, whereas levels of IL-6 were very low (16). Conversely, in the present studies in WT mice, levels of IL-10 in BAL were very low, and IL-6 levels were more predictive of lung injury (Fig. 3C). Levels of IL-6 were also found here to be important in distinguishing between the responses of WT mice and MCP-1(−/−) mice following CASP aspiration (Fig. 8B). In both mice and rats, levels of MCP-1 in BAL correlate statistically with the severity of aspiration lung injury, and this is enhanced by consideration of IL-6 levels in mice and IL-10 levels in rats. Additionally, in patients with ARDS (30), a strong correlation has been reported between levels of MCP-1 in BAL and enhanced monocytic recruitment and the severity of respiratory failure. In recent studies, our laboratory (16) has shown that treatment of rats with anti-IL-10 antibodies before CASP aspiration led to increased inflammatory lung injury, indicating the functional importance of this cytokine in modulating aspiration injury in that species. Pretreatment of rats with neutralizing antibody against MCP-1 did not have a pronounced effect on lung injury severity (16). However, administration of neutralizing antibodies is not equivalent to studying MCP-1(−/−) animals, and species differences between rats and mice may also affect comparisons with the current study.

In summary, this study has defined a murine model of aspiration lung injury from tracheally instilled ACID, SNAP, or CASP. Aspiration of CASP in WT mice led to increased albumin concentrations, increased leukocyte numbers, and increased levels of inflammatory mediators in BAL compared with the component injuries of either ACID or SNAP alone, a conceptually similar pattern to that shown previously in rats. MCP-1(−/−) mice had significantly greater mortality than WT mice when given CASP containing gastric particle levels of 10–40 mg/ml. Surviving MCP-1(−/−) mice had elevated BAL levels of KC (40 mg/ml CASP) and IL-6 (5 mg/ml CASP) compared with WT mice, without striking differences in lavaged albumin concentrations or leukocyte numbers. Lung tissue from MCP-1(−/−) mice had more severe and diffuse lung injury on histological examination, with greatly reduced ability to compartmentalize inflammatory injury compared with WT mice as assessed by granuloma formation at 24 h. These results indicate that MCP-1 plays important roles in survival and in the modulation of inflammation in severe gastric aspiration pneumonitis.

Fig. 9. Light micrographs of stained lung tissue sections from MCP-1(−/−) and WT mice at 24 h after aspiration of 40 mg/ml CASP. A: WT mouse; B: MCP-1(−/−) mouse. Representative hematoxylin and eosin-stained tissue sections at 24 h are shown. Organizing areas of injury with granuloma formation (arrowhead) is seen in WT mice compared with more severe diffuse broncho-pneumonia without granuloma formation in MCP-1(−/−) mice. All MCP-1(−/−) mice given 40 mg/ml CASP failed to survive to 48 h (Fig. 5). Magnification ×4.
GRANTS

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