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NEUTROPHIL SEQUESTRATION IN LIVER AND LUNG IS DIFFERENTIALLY REGULATED BY C-X-C CHEMOKINES DURING EXPERIMENTAL PERITONITIS[1]

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Abstract—C-X-C chemokines play an important role in the migration and activation of neutrophils (PMNs) during an inflammatory event. We measured mRNA and protein expression of the murine C-X-C chemokines macrophage inflammatory protein-2 (MIP-2) and KC in the lungs, liver, blood, and peritoneal cavity of Swiss Webster mice after cecal ligation and puncture (CLP). Neutralizing antibodies to MIP-2 and KC were also used to determine the biological effects of these chemokines on neutrophil sequestration and organ injury in the CLP model. The data showed that early after CLP, MIP-2 mRNA and protein were expressed predominantly by the lung, whereas KC mRNA and protein were expressed by the liver. Inhibition of MIP-2 reduced both lung neutrophil sequestration and peritoneal neutrophil migration. Inhibition of KC had no effect on overall neutrophil sequestration in liver but reduced injury as measured by serum transaminases. An early survival benefit was found with anti-KC treatment, although overall survival was not different. Our study showed a differential expression by organs of C-X-C chemokines during sepsis and suggested that such chemokine effects are tissue-specific.

INTRODUCTION

Abdominal sepsis remains a leading cause of morbidity and mortality among surgical patients. Multiple organ failure or dysfunction secondary to a systemic

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inflammatory response (1, 2) is now the chief cause of mortality and morbidity. This change can be related to current therapeutic measures that have efficiently dealt with overwhelming infection. The neutrophil, an essential component of the host defense mechanism (3), is also implicated in organ failure.

Understanding of the mechanisms that control neutrophil (PMN) sequestration at different anatomical sites during peritonitis could identify differences that may be manipulated to prevent organ injury without compromising peritoneal defense. In response to an inflammatory stimulus, rapidly streaming PMNs in the circulation slow down (step of rolling), firmly adhere to endothelium (step of firm adhesion), and finally migrate across the endothelium (step of transmigration). Studies have shown that this multistep process depends on both PMN and endothelial factors (4). We have previously shown (5, 6) that PMN sequestration or migration into different tissues after cecal ligation and puncture (CLP) involves a differential regulation of adhesion molecules. Blockade of P-selectin or CD18 markedly attenuated PMN influx into the peritoneal cavity, whereas PMN sequestration in the remote organ was unaffected. An equally important part of PMN-endothelial interaction is the formation of PMN chemoattractants such as C-X-C chemokines, which upregulate PMN adhesion molecules, increase respiratory burst, and form chemical gradients. Our goal was to study the pattern of regulation of the C-X-C chemokines in the lung and liver after CLP.

The C-X-C chemokines are peptides with a conserved cysteine residue near the amine terminus (7). C-X-C chemokines identified in humans include interleukin-8 (IL-8) and GRO-$\alpha/\beta/\gamma$ (8). The protein IL-8 is not found in rodents, and the identified C-X-C chemokines are functional homologues of human GRO proteins. Macrophage inflammatory protein-2 (MIP-2) (9, 10), mouse granulocyte chemotactic protein-2 (a recently described C-X-C chemokine), and KC in mice are the GRO homologues (11). Murine KC was one of the first platelet-derived growth factor-inducible competence genes to be identified and described (10). In rats, the functional homologues are the cytokine-induced neutrophil chemotactic proteins (CINC-1, 2α, 2β, and 3) (12). Through a heparin-binding domain, chemokines are able to bind to endothelial cell proteoglycans and are presented to neutrophils rolling along the endothelium (13). The subsequent activation of the chemokine transmembrane receptor causes upregulation of CD11b/CD18 on the neutrophil and increases respiratory burst (14). CD11b/CD18 binds with avidity to its endothelial ligand, intercellular adhesion molecule-1 (ICAM-1), resulting in firm endothelial adhesion that precedes transendothelial migration in response to a chemical gradient. Subsequently, activated PMNs can cause tissue injury through the release of proteases and reactive oxygen species (15, 16).

There is a differential regulation of C-X-C chemokines (e.g., MIP-2 and KC in mice) at different sites, both in constitutive expression and induction. This regulation depends on the model of injury or infection used. In a mouse model of

viral keratitis, MIP-2 appeared to be the dominant and important chemokine, and blockade of MIP-2 (unlike blockade of KC) reduced the incidence and severity of keratitis (17). On the other hand, in a mouse model of *Klebsiella* pneumonia, compartmentalized overexpression of KC improved survival (18). This effect, and the fact that KC is maximally expressed earlier on in the wild-type mice, suggested that KC was more important in the early host defense than MIP-2 in this model and was expressed much later. In a hepatic ischemia-reperfusion model in mice, both KC and MIP-2 were shown to be important, and the blockade of either chemokine reduced liver injury significantly (19). In a rat model of airway inflammation caused by fungal spores, a differential response in the induction of the same chemokine was shown to occur with different organisms and also concentrations of organisms (20). In this model, some organisms had no effect on MIP-2 (or KC) expression, whereas maximal expression of MIP-2 occurred at 1 h with certain organisms and later (1–3 h) with others. We therefore hypothesized that after CLP in mice, MIP-2 and KC would be expressed in the liver, lung, and peritoneal cavity, and would differentially control PMN sequestration at these sites.

MATERIALS AND METHODS

Care of Animals. Adult male Swiss Webster mice (age 6–8 weeks, weighing 25–30 g, Taconic, Germantown, New York) were used. Mice were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care and provided with food and water ad libitum. Studies were carried out according to the National Institutes of Health guidelines and under the supervision of a veterinarian.

Cecal Ligation and Puncture Model. Mice were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg) by intramuscular injection. CLP was performed as described previously (21). Briefly, the cecum was exposed through a midline laparotomy incision, ligated just below the ileocecal junction with 4–0 silk, and punctured twice using an 18-gauge needle. The cecum was then returned into the peritoneal cavity, and the abdominal incision was closed with 4–0 nylon suture.

Trial Design. To investigate the expression of MIP-2 and KC, 18 mice underwent CLP. At 3, 6, and 18 h, 6 mice were sacrificed. Four mice (unmanipulated) served as normal controls. Liver and lung were harvested for mRNA quantitation and protein expression as determined by enzyme-linked immunosorbent assay (ELISA). Serum and peritoneal fluid protein levels were also determined by ELISA. Mice then received intravenous anti-MIP-2 (N = 6), anti-KC (N = 6) antibody, or control serum at the time of CLP. Six hours after CLP, mice were sacrificed. The 6 h time point was chosen based on organ KC and MIP-2 expression as detected in the first experiment (*vide infra*). Liver and lung were harvested for myeloperoxidase (MPO) quantitation, serum for transaminase levels, and peritoneal fluid for differential leukocyte counts. Lastly, a group of mice received anti-KC antibody (N = 18) or control serum (N = 18) at the time of CLP, and survival was determined.

Antibodies. Polyclonal anti-murine MIP-2 antibody (BioSource, Camarillo, California) was injected at a dose of 3.3 mg/kg in 0.2 mL saline. Polyclonal rabbit anti-murine KC antibody (1 : 1000 titer) was injected at a dose of 150 μL of the anti-KC and 600 μL of normal saline. Control serum was used in a similar dilution (1 part control serum to 4 parts of normal saline).

Lung and Serum Chemokine Assays. To measure liver and lung MIP-2 and KC protein expression, tissues from the respective organs were removed, weighed, and homogenized in a lysis buffer containing 0.5% Triton X-100, 10 mM Tris, 0.5 mM $MgCl_2$, 22 μg/mL aprotinin, 100 μg/mL leupeptin, 1.8 mg/mL iodoacetamide, and 1 mM PMSF. Homogenates were incubated on ice for 30 min, centrifuged at 2500 rpm for 10 min, and supernatants were stored at −20°C until cytokine levels were determined. Liver, lung, serum, and peritoneal MIP-2 levels were estimated by using an ELISA kit (BioSource, Camarillo, California), according to the manufacturer's instructions. KC concentration was determined by ELISA, as previously described (22). Briefly, plates were coated with capture antibody overnight and blocked for 1 h on the Nutator mixer (Becton Dickinson, Cockeyville, Maryland) before samples and standards were added. The samples and standards were also placed on the Nutator for 1 h. Biotinylated antibody was then added for 1 h followed by streptavidin-peroxidase (Jackson Laboratories, West Grove, Pennsylvania) for 1 h. Both reactions occurred on the Nutator at room temperature. Peroxidase substrate was then added, and plates were allowed to develop for 20 min in the dark before the reaction was stopped with 3M H_2SO_4. Plates were read at dual wavelengths of 490 nm and 630 nm. Sample concentrations were determined by comparison to a standard curve (both proteins from R&D Systems, Minneapolis, Minnesota). The antibody used was a polyclonal antibody with specificity for KC.

Total Tissue RNA Extraction. Tissue extraction was performed according to the method described by Chomczynski and Sacchi (23) using the right lung, liver tissue, and peritoneal cell pellet.

Semi-Quantitation of MIP-2 and KC mRNA. This is a semi-quantitative competitive differential polymerase chain reaction (PCR)-based protocol described previously (24, 25). Total cellular RNA (400 ng) was reverse-transcribed according to the Geneamp RNA PCR kit (Perkin-Elmer, Norwalk, Connecticut) protocol using random hexamers to prime the reverse transcriptase. For PCR of the cDNA, the same buffer with 2.5 units of AmpliTaq DNA polymerase and a final concentration of Mg^{2+} 3 mM was used. The primers for murine beta-actin (β-actin) and either KC or MIP-2 (Stratagene, La Jolla, California) were added in the same tube at a final concentration of 0.5 mM each. We designed the MIP-2 primer sequences by using OLIGO software (Genbank accession number for murine MIP-2, X53798). The KC primer sequences were obtained from a previous study described by Oquendo, et al. (26). The primer sequences were as follows:

β-actin sense:	GTGGGCCGCTCTAGGCACCA
antisense:	CGGTT-GGCCTTAGGGTTCAGGGGGG (product size 245 bp)
MIP-2 sense:	GAACAAAGGCAA-GGCTAACTGA
antisense:	AACATAACAACATCTGGGCAAT (product size 204 bp)
KC sense:	CGGAATTCGCCACCAGCCGCCTG
antisense:	CGTCTAGACTTTCT-CCGTTAC-TTGG (product size 252 bp)

After a 2 min initial melting at 95°C, the mixture was amplified for 29 cycles, using a 3-step protocol with melting at 95°C for 1 min, annealing at 59°C for 90 sec, followed by extension at 72°C for 15 sec. The final cycle was followed by a 10-min soak at 72°C. To ensure that the amount of cytokine cDNA was measured only during the exponential phase of the PCR amplification, the optimum number of PCR cycles was determined for each primer pair before amplification. To ensure that there was no amplification of contaminating genomic DNA, control samples were run for each primer pair without the addition of reverse transcriptase. The ratios for KC to β-actin and MIP-2 to β-actin were calculated from densitometric analysis of negative films for each individual specimen, following electrophoresis and ethidium bromide staining of the PCR products. The size of the fragment was confirmed with molecular weight markers. Data are presented as ratio to β-actin.

Peritoneal Lavage. Peritoneal exudate cells were recovered in all groups by peritoneal lavage with 4 mL of ice-cold, heparinized, RPMI 1640 medium (GIBCO/BRL, Bethesda, Maryland). For differential leukocyte counts, cells were counted manually by hemocytometer, and cytospins were

performed. Cells were stained with Wright's and Giemsa stain and differentially counted. Two hundred cells were counted for each slide in a blinded fashion. For cell RNA extraction, total nonadherent leukocytes were used and extraction performed as described above. Peritoneal MIP-2 levels were estimated by using an ELISA kit (BioSource, Camarillo, California), according to the manufacturer's instructions.

Serum Transaminases. Serum was assayed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using colorimetric assays (Sigma Chemical Company), according to the manufacturer's instructions. Serum transaminases are expressed as Sigma-Frankel units/mL.

Myeloperoxidase (MPO) Assay. This was performed as previously described (27). Briefly, tissues from the right lower lung and right lobe of the liver were removed, MPO extracted, and amounts determined spectrophotometrically.

Statistical Analysis. Survival was analyzed using Fisher's exact test and differences in mRNA quantitation using Kruskal-Wallis test. Differences in chemokine protein levels, leukocyte numbers, serum transaminases, and MPO levels were analyzed using ANOVA. The null hypothesis was rejected at the $P < 0.05$ level.

RESULTS

MIP-2 and KC mRNA Expression. There was a differential expression of the C-X-C chemokine mRNA depending on the anatomic site examined. In the liver, an early increase in expression of KC mRNA was found, followed by a later increase in expression of MIP-2 mRNA (Table 1). MIP-2 mRNA was significantly expressed in the lung increasingly at 3 h and longer, whereas KC mRNA was only expressed significantly at 18 h. Early after CLP, MIP-2 mRNA

Table 1. MIP-2 and KC mRNA Levels After Cecal Ligation and Puncture (CLP)

	Liver	Lung	Peritoneal Cells
MIP-2 mRNA (Ratio to β-actin)			
Normal	0.08 ± 0.010	0.00 ± 0.000	0.05 ± 0.001
3 h	0.17 ± 0.023	0.22 ± 0.022[a]	0.40 ± 0.021[a]
6 h	0.48 ± 0.069[a]	0.66 ± 0.044[a]	0.54 ± 0.045[a]
18 h	0.57 ± 0.080[a]	0.43 ± 0.020[a]	ND[b]
KC mRNA (Ratio to β-actin)			
Normal	0.00 ± 0.000	0.13 ± 0.053	0.02 ± 0.001
3 h	0.08 ± 0.011[a]	0.22 ± 0.042	0.03 ± 0.012
6 h	0.23 ± 0.039[a]	0.22 ± 0.052	0.20 ± 0.038[a]
18 h	0.56 ± 0.125[a]	0.44 ± 0.117[a]	ND[b]

[a] $P < 0.005$ as compared to normals (Kruskal-Wallis test).
[b] ND, not done.

Table 2. MIP-2 and KC Protein Levels After Cecal Ligation Puncture (CLP)

	Liver (ng/gm)	Lung (ng/gm)	Serum (ng/mL)	Peritoneum (ng/mouse)
MIP-2 protein				
Normal	2.15 ± 0.226	0.26 ± 0.151	0.06 ± 0.037	0.06 ± 0.048
3 h	3.24 ± 0.481	2.04 ± 0.386	0.47 ± 0.165	2.27 ± 0.389[a]
6 h	5.45 ± 0.901	13.89 ± 1.322[a]	1.44 ± 0.187[a]	6.18 ± 0.844[a]
18 h	9.70 ± 2.760[a]	11.85 ± 1.940[a]	3.97 ± 0.302[a]	ND[b]
KC protein				
Normal	0.04 ± 0.001	0.32 ± 0.080	0.04 ± 0.009	ND[b]
3 h	0.07 ± 0.022	0.31 ± 0.088	0.10 ± 0.016	ND[b]
6 h	0.34 ± 0.106	1.67 ± 1.019	0.60 ± 0.282	ND[b]
18 h	5.63 ± 1.396[a]	4.33 ± 2.358	3.08 ± 0.465[a]	ND[b]

[a] $P < 0.005$ as compared to normals (ANOVA).
[b] ND, not done.

was detected in nonadherent peritoneal leukocytes, and KC mRNA was not. Only at 6 h after CLP was there evidence of an increase in KC mRNA from peritoneal leukocytes.

MIP-2 and KC Protein Expression. The pattern of MIP-2 and KC protein levels after CLP in the liver and lungs was similar to their respective mRNAs (Table 2). MIP-2 protein was found early in the lung after CLP and in increasing amounts at later time points, coinciding with lung neutrophil sequestration. Although KC protein was detected in the lung after CLP (particularly later on), these levels were not significantly greater than control animals at any of the time points measured. However in liver, both KC and MIP-2 protein levels were found to be significantly high at 18 h, but the KC mRNA was expressed earlier than MIP-2 mRNA (Table 1). Serum levels of MIP-2 were higher early on, as compared with KC, which was noted to be significantly increased 18 h after CLP. When the levels of MIP-2 in the peritoneal cavity at 6 h were compared with serum levels at this time, there was an increasing gradient from the blood to the peritoneal cavity.

Effect of Neutralizing Antibodies to KC and MIP-2

Peritoneal neutrophil migration: At 6 h after CLP, there was a significant increase in the number of neutrophils in the peritoneal cavities of mice injected with control serum compared to unmanipulated controls (Figure 1). Although anti-KC antibodies reduced peritoneal neutrophil migration compared with control serum mice, this difference was not significant. In contrast, anti-MIP-2 anti-

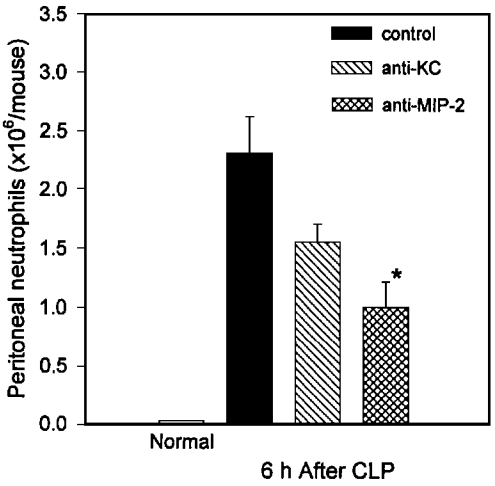

Fig. 1. Peritoneal neutrophil migration 6 h after cecal ligation and puncture (CLP) with anti-KC and anti-MIP-2 treatment. $*P < 0.05$ as compared to normals (ANOVA).

body administration significantly reduced peritoneal neutrophil migration. This time point corresponds to an increase in both MIP-2 mRNA expression (Table 1) and peritoneal exudate MIP-2 protein (Table 2).

MPO: Both liver (Figure 2) and lung MPO (Figure 3) increased signifi-

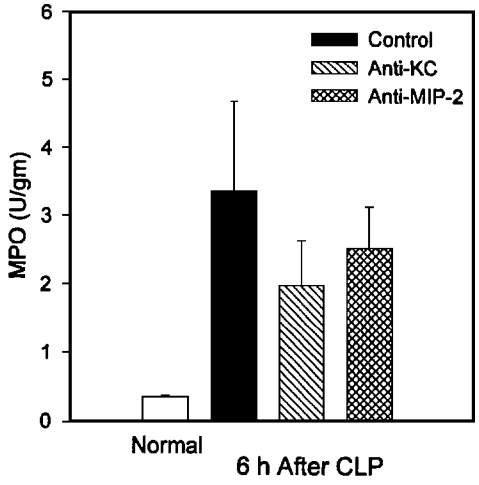

Fig. 2. Liver MPO 6 h after cecal ligation and puncture (CLP) with anti-KC and anti-MIP-2 treatment.

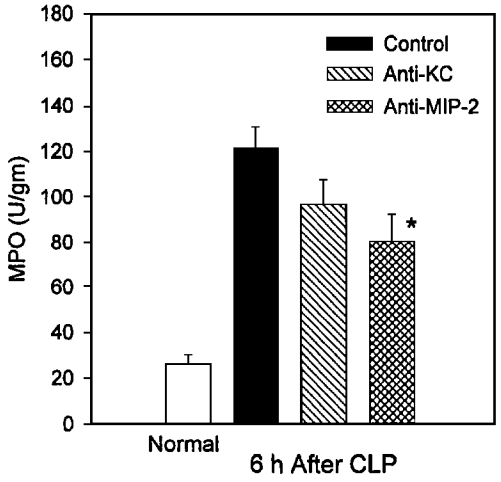

Fig. 3. Lung MPO 6 h after cecal ligation and puncture (CLP) with anti-KC and anti-MIP-2 treatment. $*P < 0.05$ as compared to control (ANOVA).

cantly at 6 h after CLP as shown by serum controls when compared with normal controls. Inhibition of MIP-2 significantly reduced lung MPO compared with serum controls, whereas inhibition of KC had no effect. At this 6-h point, MIP-2 protein levels in the lung were significantly elevated, but those for KC were not (Table 2). In the liver at 6 h, KC protein levels were elevated but not significantly (Table 2), and even though inhibition of KC reduced liver MPO, this did not achieve statistical significance (Figure 2). Inhibition of MIP-2 had little effect on liver MPO.

Serum transaminases: Both serum ALT and AST levels were increased after CLP (Table 3). Inhibition of MIP-2 had no effect on either transaminase

Table 3. Serum Levels of Liver Enzymes

	AST SFU[a]/mL	ALT SFU[a]/mL
Normal	48 ± 5.2[b]	23 ± 2.2[b]
Control IgG	211 ± 15.4	125 ± 17.8
Anti-MIP-2	193 ± 24.8	113 ± 8.5
Anti-KC	145 ± 10.2[b]	80 ± 5.0[b]

[a]SFU, Sigma-Frankel units.
[b]$P < 0.005$ as compared with control IgG (ANOVA).

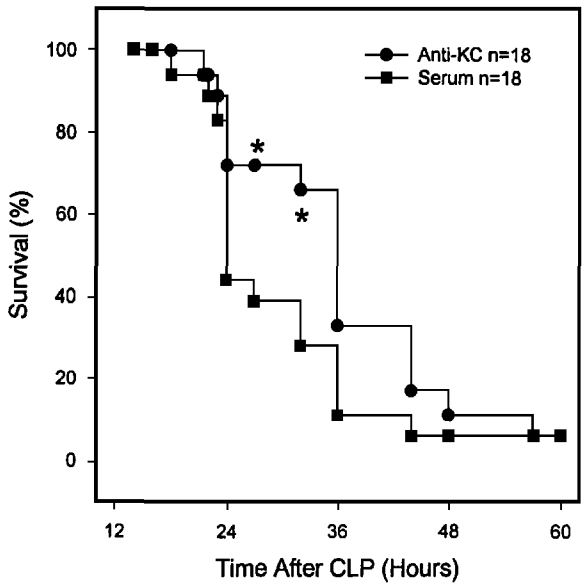

Fig. 4. Survival after 18-gauge, double-puncture cecal ligation and puncture (CLP) with anti-KC treatment. $*P < 0.05$ as compared to serum controls (Fisher's exact test).

level. Interestingly, inhibition of KC significantly reduced both transaminases as compared with control.

Survival After CLP With Anti-KC Treatment. As shown in Figure 4, anti-KC administered at the time of CLP significantly improved early, but not overall survival.

DISCUSSION

There is evidence to suggest that the human C-X-C chemokine IL-8 plays a pivotal role in the pathogenesis of PMN-mediated adult respiratory distress syndrome, multiple organ failure, and death in septic patients (28, 29). The clinical observations are supported by experimental work in rabbits, which produce IL-8 similar to humans. Neutralizing antibodies to IL-8 in a rabbit endotoxemia model inhibited lung neutrophil sequestration, improved pulmonary function, and improved survival (30). The importance of the C-X-C chemokines in causing organ PMN sequestration has been demonstrated by gene transfer studies. The transfer and overexpression of MIP-2 in rat lungs and of CINC-1 in rat livers (31, 32) resulted in local organ PMN sequestration and organ injury in the case of CINC-1. Transgenic mice overexpressing KC in lungs have been shown to

have significantly higher survival from pneumonia compared to wild-type mice, as well as decreased bacterial recovery from the lung and blood. Increased PMN influx into the lungs in these animals was also observed (18).

The current study demonstrates that KC mRNA and protein are not constitutively expressed in the liver, and KC mRNA is produced early on following CLP. This coincides with hepatic PMN sequestration, and these results are consistent with those of Deutschman et al. (33), who demonstrated an early increase in CINC mRNA in hepatocytes and Kupffer cells (and CINC protein in liver homogenates) after CLP in rats. In a hepatic ischemia-reperfusion model in mice, KC was induced early after injury in the liver. MIP-2 mRNA is induced later on in this model (19). In our study, in contrast to KC, hepatic MIP-2 mRNA and protein were constitutively expressed in small amounts, and then produced by 6 h after CLP, but protein-expression did not reach significant levels before 18 h. A similar observation was noted by Walley et al. (34), who observed a small, but significant increase in liver MIP-2 protein levels in CD1 mice subjected to CLP. They also observed a much greater expression of MIP-2 protein in the lung and peritoneum compared to the liver after CLP. The importance of KC versus MIP-2 in the causation of liver injury is illustrated by the reduction in serum transaminases after neutralization of KC, but not of MIP-2 after CLP. Interestingly, the reduction in transaminases was achieved without an overall reduction in liver PMN sequestration. It has been shown that transendothelial migration rather than sinusoidal sequestration alone of primed PMNs is responsible for hepatic parenchymal damage (35). The method we used for quantifying liver PMN sequestration, the MPO assay, has been shown by us (5) and others to concur with histological assessment of both sinusoidal *and* hepatic parenchymal PMN sequestration. However, the liver was not flushed of blood in this study, and it is plausible that there was an actual reduction in transendothelial migration of PMNs in the anti-KC-treated mice.

In endotoxemic rats treated with anti-CINC antibody, a reduction in both sinusoidal and hepatic parenchymal PMN sequestration was observed (36). However, organ injury and death in the CLP model are independent of endotoxemia, so other mechanisms may be involved in the endotoxemia model that probably reflect the direct tissue toxicity of endotoxin or the overwhelming systemic proinflammatory cytokine response to systemically administered endotoxin (37). Another explanation for the reduction in liver injury is based on the observation that the cytotoxic potential of activated PMNs depends on their adherence to hepatocytes, and that this can be CD18-mediated (38, 39). Therefore, the attenuation in transmigrating PMNs may be responsible for reducing the actual hepatic injury. C-X-C chemokines, in turn, upregulate CD18 on PMNs (14), and in an immune complex-induced lung injury model in rats, inhibition of CINC reduced bronchoalveolar PMN migration as well as CD18 expression on these PMNs (40). In contrast, in the rat endotoxemia model mentioned above, there

was no reduction in CD18 on isolated hepatic PMNs after anti-CINC treatment (36). This may indicate that the role of chemokines is differentially regulated, depending on the animal model used. We have previously shown no reduction in liver PMN sequestration or hepatic injury in anti-CD18-treated mice subjected to CLP (5). In vivo and in vitro data suggest that PMN-mediated hepatic injury is both reactive oxygen- and protease-dependent, with reactive oxygen species potentiating protease injury (41, 42). KC has also been shown to be a potent stimulator of reactive oxygen species from PMNs (14), and thus, one further explanation may be that the anti-KC antibody reduced PMN cytotoxicity by blockade of this activity. The survival data with anti-KC treatment suggest a benefit in the early time period after CLP, although there was no significant difference in overall survival between the two groups. Blockade of MIP-2 and KC does not completely abrogate the hepatic injury in a murine ischemia-reperfusion model (19). This further implies that there are other factors in the inflammatory cascade that contribute to the injury as well.

In contrast to the expression of MIP-2 mRNA and protein observed in the liver after CLP, both MIP-2 mRNA and protein in the lung were significantly elevated at earlier time points after CLP, coinciding with lung PMN sequestration. Also unlike in the liver, inhibition of MIP-2 reduced lung PMN sequestration. The effect of neutralization of MIP-2 on survival was not tested since it has already been shown that anti-MIP-2 antibody administered prior to CLP significantly improved survival (34). This group did not measure lung PMN sequestration, and our results suggest that improvement in survival could be due to reduction in PMN-mediated lung injury. We have shown that lung injury after CLP is, in part, PMN-mediated (2). In that study, the use of an anti-PMN antibody reduced such lung injury. Our observations are supported by those of Schmal et al. (43), but their model was one of direct intratracheal instillation of endotoxin in rats. They found a time-dependent increase in MIP-2 mRNA and protein and a similar decrease in lung MPO. In addition, they found a significant reduction in pulmonary vascular permeability in anti-MIP-2-treated rats. As with liver sinusoidal PMN sequestration, PMN arrest and subsequent transendothelial migration in the lung occurs predominantly in capillaries (44). In that scenario, PMN adhesion and migration may use different adhesion pathways than in the systemic circulation, with physical constraints caused by lack of PMN deformability and endothelial swelling being more relevant than selectin and (chemokine-induced) integrin-mediated adhesion. Since lung PMN sequestration is CD18-independent after CLP (6), the observed reduction in lung MPO in anti-MIP-2-treated mice suggests that MIP-2 (produced predominantly by alveolar macrophages) acts more as a chemoattractant than stimulator of adhesion molecules. This mechanism may not be the principal mode of action in the peritoneal cavity, since we have shown that peritoneal PMN migration is both P-selectin- and CD18-dependent after CLP (5, 6). Thus, MIP-2 may function as both an upregulator

of CD18 and chemoattractant in the migration of peritoneal PMNs after CLP. The lack of production of KC in the lung after CLP and failure to reduce PMN sequestration after CLP with anti-KC antibody administration contrast to findings of other investigators using different models of injury. Intratracheal administration of endotoxin in a rat model showed that CINC was produced within the lung, and that its neutralization reduced lung PMN accumulation (14). In the *Klebsiella* pneumonia model in mice (which also used transgenic mice with lung-specific overexpression of KC), KC appeared to be more important than MIP-2 in the early PMN influx in the lung (18). When CLP was performed in endotoxin-resistant and endotoxin-sensitive mice, we found no difference in lung KC mRNA expression, but rather a reduction in MIP-2 mRNA expression and lung PMN accumulation in the endotoxin-resistant mice was noted (37). Thus, it appears, that PMN migration into both liver and lung is differentially regulated, depending on the inciting stimulus.

In early peritoneal PMN migration after CLP, MIP-2 appears to be more important than KC. This is evident by the earlier and more significant rise in the MIP-2 mRNA and protein levels that were noted, and the significant attenuation of the PMN influx using anti-MIP-2 antibodies and not anti-KC antibodies. We have shown previously (45) that this action of MIP-2 is significantly dependent on peritoneal mast cells and also the expression of P-selectin.

These results suggest a differential modulation in the expressions and functions of C-X-C chemokines, depending on the nature of the inciting stimulus, the type of injury produced, the tissue studied, as well as the animal model used for study. More importantly, a redundancy in the chemokine response is evident from all of these studies. We believe the CLP model more accurately reflects human peritonitis and organ injury and is, therefore, more relevant to the corresponding human scenario of acute abdominal sepsis.

In summary, the anti-inflammatory effects of anti-KC in the liver and anti-MIP-2 in the lung in this model of abdominal sepsis are the most effective therapies we have observed. In this model, we have previously shown that neither anti-adhesion molecule (P-, E-, L-selectins, or CD18) nor anti-cytokine (tumor necrosis factor or IL-1) therapy have reduced organ injury or PMN sequestration. The effects of anti-KC in reducing hepatic injury and improving early survival without compromising peritoneal host defense deserve further study and argue for more characterization of C-X-C chemokine function in the corresponding human clinical scenario. The differential modulation of expression and functions of the C-X-C chemokines in different experimental models also underscores the importance of clinically relevant animal models to accurately translate such data to specific human disease states.

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