

# Ratio of Local to Systemic Chemokine Concentrations Regulates Neutrophil Recruitment

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**CXC chemokines are important regulators of local neutrophil recruitment. In this study, we examined the role of the ratio of local to systemic chemokine concentrations as a significant factor determining local neutrophil recruitment. Thioglycollate was injected intraperitoneally into BALB/c mice resulting in a dose-dependent increase in neutrophil recruitment and local inflammation, as measured by peritoneal levels of interleukin 6. At the high dose of 3% thioglycollate, antibody inhibition of the murine chemokines KC and macrophage inflammatory protein-2 caused a reduction in peritoneal neutrophil recruitment by as much as 93%. A paradoxical effect was observed with a 0.3% thioglycollate intraperitoneal challenge. In this situation, inhibition of KC resulted in a significant increase in peritoneal neutrophils, and inhibition of macrophage inflammatory protein-2 also resulted in increased peritoneal neutrophils. These results were consistent with a reverse chemotactic gradient as described by the ratio of peritoneal to plasma KC levels. A higher ratio (ie, increased peritoneal chemokines compared to plasma) resulted in increased neutrophil recruitment after either the 3% or 0.3% thioglycollate challenge. Our results demonstrate that whereas sufficient local concentrations of chemokines are necessary, a critical factor dictating local neutrophil recruitment is the ratio of the local to the systemic chemokine concentrations. (Am J Pathol 2001, 158:715–721)**

Neutrophils are involved in a large number of pathological conditions but they play a particularly important role during acute inflammation. These cells actively defend against pathogenic organisms and foreign bodies by phagocytosis and by releasing a host of specific and nonspecific bactericidal compounds.<sup>1,2</sup> Nonspecific defense mechanisms (eg, release of free radicals) also damage host tissue and this damage can be considerable in some disease states. Neutrophil activation and trafficking seem to be regulated by chemokines.<sup>3</sup> Consequently, a better understanding of how different che-

mokines affect neutrophils is critical to identifying mediators of acute inflammation.

Several groups of chemokines have been identified based on the relative position of two conserved cysteine residuals (C, CXC, CX3C).<sup>3</sup> CXC chemokines that incorporate an ELR motif (ELR+) near the N-terminus are particularly potent inducers of neutrophil chemotaxis. In humans, seven ELR(+)CXC chemokines have been identified, of which interleukin (IL)-8 seems to be the most important neutrophil attractant and activator during acute inflammation.<sup>3–5</sup> Studies of inflammation necessarily involve animal models, and murine models have been used extensively in this context. Nevertheless, no structural homologue for IL-8 has been identified in mice. Although several murine ELR(+)CXC chemokines have been identified to date [ie, KC, macrophage inflammatory protein (MIP-2), and LIX], they are structurally homologous to human chemokines other than IL-8.<sup>6</sup>

During an inflammatory event, concentrations of cytokines typically increase more at the local site of inflammation compared to systemic levels.<sup>7,8</sup> At a compartmental level (eg, peritoneum), this has not necessarily been true for murine chemokines. In a murine cecal ligation and puncture model, Walley and colleagues<sup>9</sup> found significantly elevated, but approximately equal concentrations of peritoneal and serum MIP-2. In their studies of cecal ligation and puncture, Ebong and colleagues<sup>10</sup> also reported approximately equal increases in peritoneal lavage and plasma MIP-2, but found a >10-fold higher concentration of plasma KC compared with peritoneal lavage. These latter kinetics studies illustrated how the complexities of sepsis can decouple any clear correlations between chemokine levels and neutrophil recruitment.

In the present study we examined the *in vivo* relationship between murine chemokines and neutrophil trafficking. To avoid the complexities inherent in a sepsis model, we used a thioglycollate model of inflammation. It has long been recognized that intraperitoneal injections of thioglycollate in mice will induce rapid and abundant recruitment of neutrophils into this compartment without

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inducing degranulation.<sup>11</sup> Consequently, this represents a relatively simple model that, when combined with neutralizing antibodies for KC and MIP-2, offers a unique opportunity to gain insight into the relative contribution of individual murine chemokines to neutrophil trafficking. We also examined how KC and MIP-2 might contribute to expression of other inflammatory mediators.

## Materials and Methods

We produced recombinant KC and MIP-2 using pGEX-2T vectors (Pharmacia, Uppsala, Sweden) that incorporated a glutathione S-transferase fusion protein for column purification of the recombinant proteins. These recombinant proteins were used to generate polyclonal antibodies in rabbit and goat hosts. Our antibodies for KC and MIP-2 have demonstrated neutralizing capacity both *in vitro* and *in vivo*,<sup>12</sup> and we used them to develop highly specific and sensitive enzyme-linked immunosorbent assays (ELISAs).

## ELISAs

We developed sandwich ELISAs for KC and MIP-2 using our polyclonal antibodies. IgG was purified from rabbit sera using protein A columns (Pierce Chemical Co., Rockford, IL) and these polyclonal antibodies were used for both capture and detection. Secondary antibodies were conjugated to biotin (Pierce). Streptavidin-conjugated horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA) was used with 3,3',5,5' tetramethylbenzidine substrate (Genzyme, San Carlos, CA) for colorimetric detection and quantification. We used commercially available recombinant KC and MIP-2 for our standard curves (R&D Systems, Minneapolis, MN). This provided an independent verification of both the sensitivity and specificity of our ELISAs. Both KC and MIP-2 ELISAs were sensitive (<20 pg/ml) and showed no cross-reaction over the range of our standard curves (2 pg/ml to 20 ng/ml). Both KC and MIP-2 sandwich ELISAs were partially suppressed in the presence of plasma or serum. Consequently, when assaying plasma for these chemokines, the standard curve always contained an equivalent dilution of normal mouse plasma.

## Tumor Necrosis Factor (TNF)- $\alpha$ and IL-6 Assays

TNF- $\alpha$  bioactivity was assessed using the WEHI 164 subclone 13 bioassay<sup>13</sup> as previously described.<sup>14,15</sup> IL-6 was quantified using a B9 cell proliferation assay<sup>16</sup> following the methods of Wollenberg and colleagues.<sup>17</sup> We routinely detected <3 pg/ml of biologically active TNF- $\alpha$  and IL-6 using these bioassays in conjunction with recombinant protein standards (TNF- $\alpha$  from Cetus Immune Corp., Emeryville, CA; IL-6 from Preprotech, Rocky Hill, NJ).

## In Vivo Neutralization Experiments

Normal BALB/c mice (17 to 19 g; Harlan, Indianapolis, IN) were passively immunized by subcutaneous injection of

anti-KC (goat), or anti-MIP-2 (rabbit) or both antibodies or control serum (goat and rabbit). All serum was heat inactivated (56°C, 30 minutes) and filter-sterilized before use. Injections were composed of 333  $\mu$ l of antiserum, 333  $\mu$ l of normal saline, and 333  $\mu$ l of normal serum from goat (if anti-KC) or rabbit (if anti-MIP-2). Normal serum was not added when both antibodies were used. After 2 hours, immunized mice were lightly anesthetized with methoxyflurane (Shering-Plough, Union, NJ) and given an intraperitoneal injection of 2.0 ml of sterile thioglycollate (0.3% or 3.0%; Difco, Detroit, MI). Four hours later mice were anesthetized (ketamine, 1.7 mg/mouse and xylazine, 1.3 mg/mouse) and exsanguinated via retro-orbital plexus bleed into heparinized tubes. Plasma was stored at -20°C for later analysis. Mice were immediately euthanized via cervical dislocation and the peritoneal cavity was opened aseptically. Any residual thioglycollate was collected or if none was present, the cavity was flushed with 1.0 ml of ice-cold Hanks' buffered salt solution (no Mg<sup>+2</sup> or Ca<sup>+2</sup>; Life Technologies, Gaithersburg, MD). Cells from this initial wash were pelleted and the peritoneal supernatant stored at -20°C after recording the total volume recovered. The peritoneal cavity was then washed with 20 ml of Hanks' buffered salt solution and all cells collected from the peritoneal cavity were resuspended together. Cytospins were prepared for differential counts and total leukocytes were quantified with a Coulter counter (Coulter Corp., Miami, FL). KC and MIP-2 were quantified for the plasma and peritoneal samples, and the latter was expressed as the total amount of chemokine recovered to reflect differences in volume recovery between animals. The heart was perfused with 2 to 5 ml of normal saline and the right lung removed to quantify the level of myeloperoxidase present in the tissue.<sup>18,19</sup> This latter assay is an indicator of the relative number of neutrophils that are sequestered in the pulmonary capillary beds and serves as an indicator of neutrophil adhesion and rigidity.<sup>20</sup>

## Statistical Analyses

Comparisons between treatments and controls were made using Wilcoxon signed-rank tests. We used paired Wilcoxon signed-rank tests or Mann-Whitney *U* tests for other two-way comparisons. Pearson correlations were reported for tests of association. All statistical calculations were made using NCSS 97,<sup>21</sup> or SigmaStat (SPSS, Chicago, IL).

## Results

### Peritoneal Response to Thioglycollate

Normal mice had few neutrophils within the peritoneum and IL-6 was not detectable (Table 1). We evaluated the peritoneal response to 0.3% and 3% thioglycollate and found a dose-dependent increase in the peritoneal concentration of IL-6 and the number of recruited neutrophils (Table 1). In this limited study, a 10-fold increase in the thioglycollate concentration caused a 10-fold increase in

**Table 1.** Injection of Mice with Thioglycollate

Challenge	Peritoneal IL-6, total ng	Peritoneal PMNs, $\times 10^4$
Normal saline	<0.02	3 $\pm$ 1
0.3% thioglycollate	15 $\pm$ 3	20 $\pm$ 6
3.0% thioglycollate	149 $\pm$ 32*	234 $\pm$ 91*

Mice were injected with 0.3% or 3% thioglycollate and the peritoneal fluid and cells were collected 4 hours later.

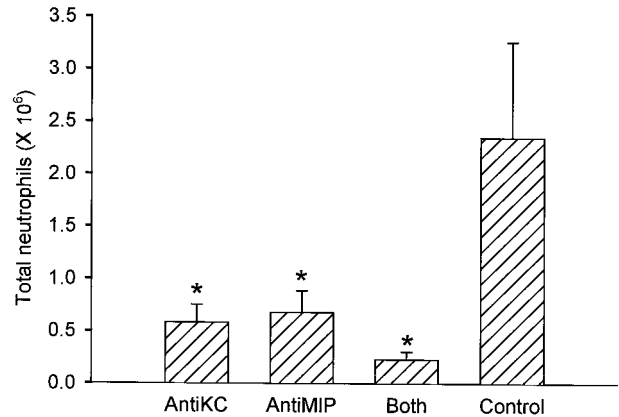
\*,  $P < 0.01$  0.3% compared to 3% thioglycollate.

$n = 6$  for normal mice, 12 for 0.3%, and 8 for 3.0%.

the IL-6 levels, and a 10-fold increase in neutrophil recruitment.

### 3.0% Thioglycollate

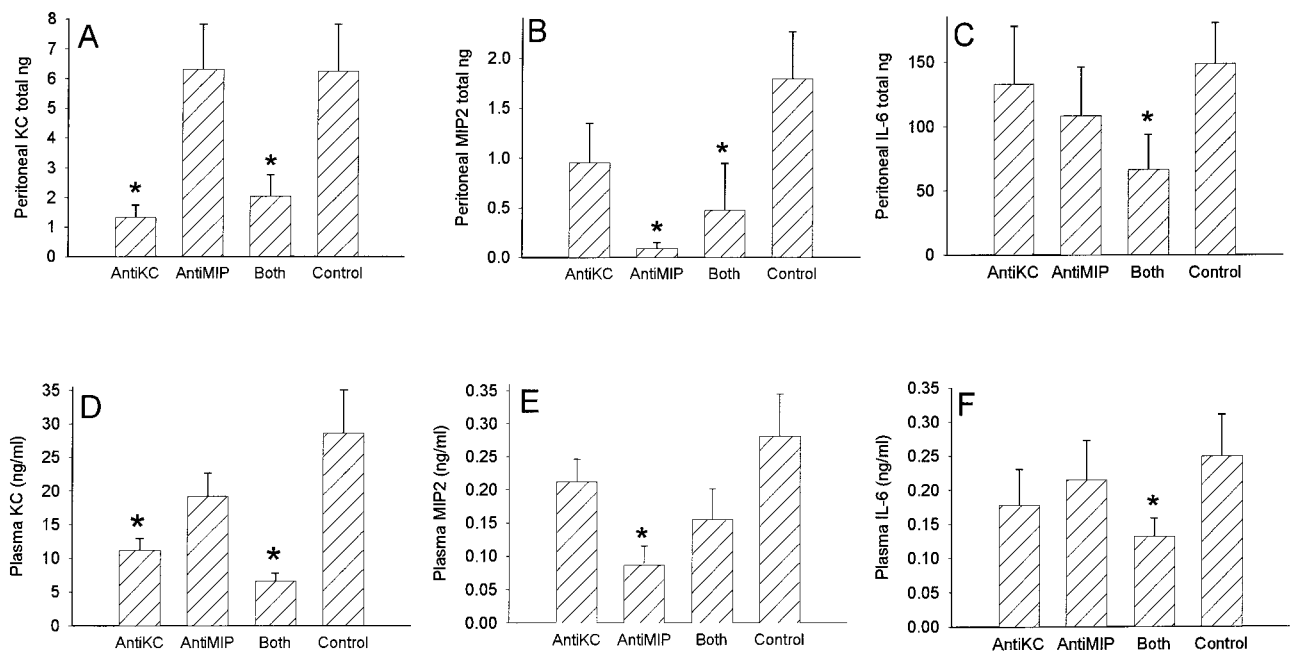
We next examined the local and systemic production of chemokines in response to the thioglycollate injections. To better understand the contributions of the individual chemokines to this recruitment, we also passively immunized mice against the murine chemokines KC and MIP-2. In normal mice, plasma and peritoneal levels of the murine chemokines are generally below detection limits (data not shown). Four hours after intraperitoneal injection of 3% thioglycollate, there was a substantial increase in both the peritoneal and plasma levels of the murine chemokines (Figure 1). Plasma and peritoneal levels of IL-6 were also increased, indicating both a systemic and local inflammatory response. Antibody inhibition of KC reduced both plasma and peritoneal levels of KC. In a similar manner, antibody inhibition of MIP-2 reduced MIP-2 concentrations both in the plasma and the peritoneum (Figure 1). As we have previously reported,



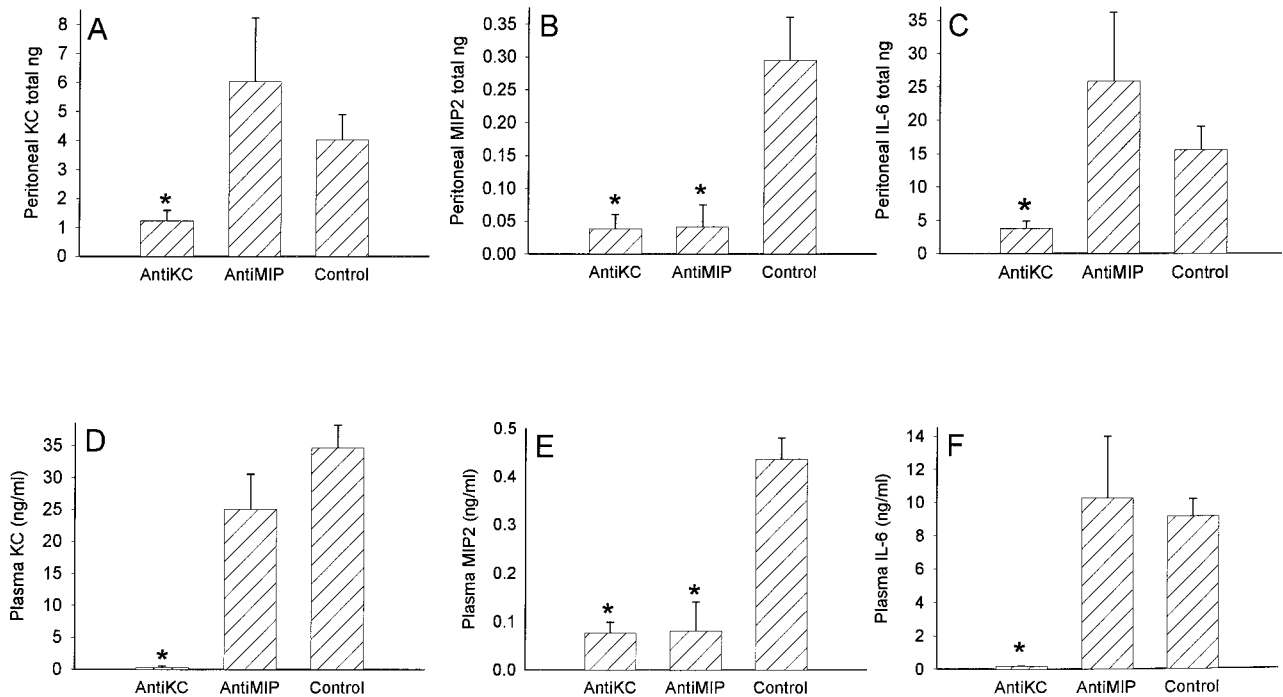
**Figure 2.** Total peritoneal neutrophils from mice challenged with intraperitoneal injection of 3% thioglycollate (2.0 ml, 4 hours). Mice were passively immunized with polyclonal antibodies against KC (AntiKC), or MIP-2 (AntiMIP), or both AntiKC and AntiMIP (Both), or with control serum (Control). Data from three independent experiments with total sample sizes of seven to nine BALB/c mice. \*,  $P < 0.05$  for Wilcoxon signed-rank test comparing treatment and control.

the levels of KC are always greater than the levels of MIP-2.<sup>10</sup> A somewhat surprising finding was that inhibition of both MIP-2 and KC caused a significant reduction in the plasma and peritoneal concentrations of IL-6. There was no TNF- $\alpha$  detected in any samples from animals treated with 3.0% thioglycollate.

The passive immunization against either KC or MIP-2 caused a significant reduction in the recruitment of neutrophils to the peritoneum (Figure 2). Blockade of KC and MIP-2 reduced neutrophil counts 80% and 76%, respectively (Figure 2). Immunization against both chemokines reduced neutrophil recruitment by 93%. Immunizing



**Figure 1.** Total peritoneal KC, MIP-2, and IL-6 (A, B, and C, respectively) recovered from mice challenged with intraperitoneal injection of 3% thioglycollate, and plasma concentrations of KC, MIP-2, and IL-6 (D, E, and F, respectively). Mice were passively immunized with polyclonal antibodies against KC, or MIP-2, or both KC and MIP-2, or with control serum. Data were from three independent experiments with a total of seven to nine BALB/c mice per treatment. \*,  $P < 0.05$  for Wilcoxon signed-rank test comparing treatment and control.



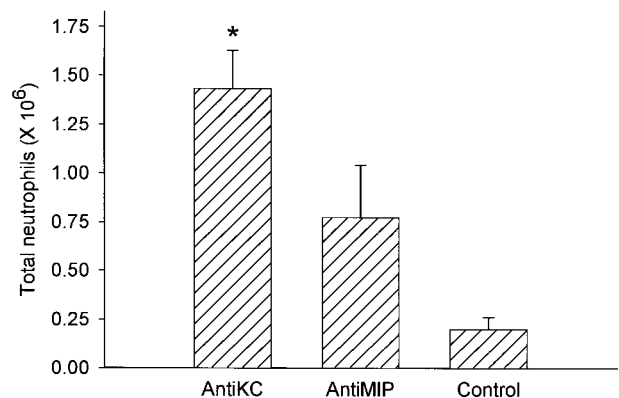
**Figure 3.** Total peritoneal KC, MIP-2, and IL-6 (A, B, and C, respectively) recovered from BALB/c mice challenged with an intraperitoneal injection of 0.3% thioglycollate, and plasma KC, MIP-2, and IL-6 (D, E, and F, respectively). Mice were passively immunized with polyclonal antibodies against KC, or MIP-2, or with control serum. Data from four independent experiments with total sample sizes of 12 mice per treatment. \*,  $P < 0.05$  for Wilcoxon signed-rank test comparing treatment and control.

against MIP-2 or against both KC and MIP-2 significantly reduced pulmonary neutrophil sequestration (data not shown).

We next examined the production of chemokines in response to the 0.3% thioglycollate intraperitoneal challenge. Similar to the results with the 3% thioglycollate, antibody inhibition of KC or MIP-2 resulted in reduced chemokine concentrations in the plasma and peritoneum (Figure 3). In this model, however, inhibition of KC alone resulted in a significant reduction in both the plasma and peritoneal levels of MIP-2. This inhibition is not because of nonspecific cross-reactivity between the anti-KC antibody and MIP-2 protein (unpublished observations). Additionally, blockade of KC resulted in significant reductions in the levels of IL-6. Again, no biologically active TNF- $\alpha$  was detectable in the specimens.

To determine the contribution of the chemokines to the recruitment of neutrophils into the peritoneum, we examined the number of peritoneal neutrophils after passive immunization against KC. In this situation, we observed the apparently paradoxical effect of increased local neutrophil recruitment with inhibition of KC (Figure 4). After passive immunization against KC, 0.3% thioglycollate treatment resulted in a sevenfold increase in the number of peritoneal neutrophils over control antibody animals. Antibody inhibition of MIP-2 also increased peritoneal neutrophil recruitment, but the results were not significant. The KC antibody inhibition also caused a significant reduction in neutrophil sequestration within the lung, as indicated by reduced pulmonary myeloperoxidase (data not shown).

Chemokines recruit neutrophils by establishing a gradient between a local compartment and the proximate, intravascular space.<sup>22</sup> In the *in vivo* thioglycollate model, this gradient may be quantified by determining the ratio between the peritoneal and plasma levels of the chemokines. Based on our results (Figure 4), KC seems to be the more important chemokine for the recruitment of neutrophils, and therefore we quantitated the ratio of peritoneal to plasma KC after either 0.3% or 3% thioglycollate challenge. This was done to test the hypothesis that the ratio of local to systemic chemokines dictates the biolog-



**Figure 4.** Total peritoneal neutrophils recovered from mice challenged with intraperitoneal injection of 0.3% thioglycollate (2.0 ml, 4 hours). Mice were passively immunized with polyclonal antibodies against KC (AntiKC), or MIP-2 (AntiMIP), or with control serum (Control). Data from four independent experiments with total sample sizes of 12 BALB/c mice per treatment. \*,  $P < 0.05$  for Wilcoxon signed-rank test comparing treatment and control.

**Table 2.** Neutrophil (PMNs) Recruitment and the Ratio of Peritoneal to Plasma KC 4 Hours after Thioglycollate Challenge

Thioglycollate	Rx	Peritoneal KC, total ng	Plasma KC, ng/ml	Ratio peritoneum: plasma	PMNs, $\times 10^5$
0.3%	Control	4.0	34.7	.11	2
0.3%	$\alpha$ -KC	1.2	0.3	4.00	14
3%	Control	6.2	28.6	.22	23
3%	$\alpha$ -KC	1.3	11.1	.12	7

ical response. As indicated in Table 2, the changes in the ratio of peritoneal to plasma chemokines observed with antibody inhibition provided an explanation for the observations of the neutrophil recruitment. After 3% thioglycollate injection, the ratio of peritoneal to plasma KC was 0.22. This ratio was reduced 50% with antibody inhibition, and the number of recruited neutrophils was reduced nearly fourfold. In contrast, for the 0.3% challenge the peritoneal to plasma ratio of KC was increased 36-fold with antibody inhibition, and the local recruitment of neutrophils was increased sevenfold. Antibody inhibition of MIP-2 reduced neutrophil recruitment for animals receiving 3% thioglycollate ( $23 \times 10^5$  to  $6.8 \times 10^5$ ). In this case we again found a reduction in the peritoneal to systemic concentrations of MIP-2 (6.4 to 1.0, respectively).

### Discussion

Our results show that both KC and MIP-2 play an important role in thioglycollate-induced peritonitis, and the local to systemic ratios of chemokines directs local recruitment of neutrophils. For the more severe model (3%), neutralization of either chemokine reduced neutrophil recruitment >75% and was consistent with reductions in both the absolute concentrations of chemokines and the local to systemic ratios of either KC or MIP-2. Simultaneously neutralizing both chemokines resulted in a 93% reduction in neutrophil recruitment. The importance of KC and MIP-2 seen in our system has been demonstrated for other models of inflammation. Walley and colleagues<sup>9</sup> showed that murine MIP-2 is significantly elevated in a cecal ligation and puncture model and passive immunization against MIP-2 resulted in a significant reduction in neutrophil recruitment.<sup>23</sup> Greenberger and colleagues<sup>24</sup> used immunization against MIP-2 to demonstrate that this chemokine is important for neutrophil recruitment and it is required to clear bacteria in a *Klebsiella* model of pneumonia. In a hepatic ischemia and reperfusion model, neutralization of MIP-2 or KC resulted in significant decreases in hepatic neutrophil accumulation.<sup>12</sup> In another model of *Klebsiella pneumoniae*, transgenic mice overexpressing KC in the lung compartment were able to recruit more neutrophils and enhance bacterial clearance.<sup>25</sup> Passive immunization against IL-8 reduced rat neutrophil recruitment 58% for a model of lung inflammation.<sup>26</sup>

Despite these studies and the work of Ebong and colleagues,<sup>10,14</sup> the precise individual chemokine contribution to the inflammatory process is not clear. Both KC and MIP-2 have been shown to elicit neutrophil chemotaxis *in vitro* and *in vivo*<sup>27-29</sup> and both proteins bind equally well to the murine homologue for IL-8 receptor

B.<sup>29,30</sup> Both KC and MIP-2 up-regulate Mac-1 on neutrophils; an important adhesion molecule involved in neutrophil chemotaxis.<sup>28,29</sup> In this study, inhibition of MIP-2 (3% challenge) or KC (0.3% challenge) reduced myeloperoxidase significantly; a result that suggests both chemokines may be involved in neutrophil stiffening subsequent to extravascular transmigration.<sup>20</sup> The high degree of functional redundancy between KC and MIP-2 may be exploited as a means for organ- and cell-specific regulation of leukocytes. For instance, tissue-specific differences were found with an endotoxin model of inflammation. KC was expressed highest in lung, heart, and liver whereas MIP-2 was mostly expressed in lung and to a lesser extent heart tissue.<sup>6</sup> KC also is constitutively expressed and may serve an important role in maintaining neutrophils outside the vascular compartment.<sup>6,12,30</sup> For our model of more severe peritonitis (3% thioglycollate), both KC and MIP-2 seemed to play significant roles in the inflammatory process, probably via redundant and distinct mechanisms.

Our less severe model of peritonitis (0.3% thioglycollate) demonstrated a more significant relationship between KC and neutrophil recruitment when one calculates the peritoneal:plasma ratio. This model, although eliciting 10-fold fewer neutrophils (compared to 3% model) still induced high local and systemic levels of KC, but reduced levels of MIP-2 and IL-6. In animals treated with control anti-sera, the high level of systemic KC relative to peritoneal KC may have disrupted the chemotactic gradient into the peritoneum. When we immunized against KC, plasma levels of KC plummeted while there was less reduction in the peritoneal KC concentration. Despite the decreased levels of the chemoattractant there still seemed to be sufficient local KC (>1 ng) to attract circulating neutrophils to the peritoneum. Consequently, it is apparent that the easily induced and very high levels of systemic KC can reverse the chemotactic gradient or otherwise interfere with chemotaxis and thus serve as an anti-inflammatory mediator in the face of minor irritants. Others have shown that administration of IL-8 will reduce neutrophil adhesion *in vitro*<sup>31</sup> and *in vivo*.<sup>32-34</sup> Ley and colleagues<sup>33</sup> concluded that high systemic levels of IL-8 inhibited transmigration of neutrophils by interfering with L-selectin-independent adhesion. Simonet and colleagues<sup>34</sup> also concluded that high systemic levels of IL-8 could interfere with adhesion, although they also addressed the possibility of a reversal of chemotactic gradients. The results of our 0.3% thioglycollate model seem to mimic the anti-inflammatory actions of the former IL-8 experiments.

A surprising finding in these studies is the observation that injection of a lower concentration of thioglycollate (0.3%) resulted in higher plasma levels of IL-6 relative to the injection of 3% thioglycollate. The lower concentration of thioglycollate did result in significantly fewer neutrophils recruited into the peritoneum, and reduced peritoneal levels of IL-6 and MIP-2. We thus have the paradoxical result in which less inflammation apparently induced higher levels of plasma IL-6. Although we do not have a definitive answer for this observation, it may be related to the timing of sampling. In a sepsis model it has been shown that the more severe sepsis results in peak plasma levels of IL-6 at a later time point.<sup>10</sup> Because all of the plasma samples were obtained at 4 hours after injection, it is possible that the higher concentration of thioglycollate would have induced greater concentrations of plasma IL-6 at later time points.

Several investigators have documented that increased local levels of chemokines are associated with increased recruitment of inflammatory cells. In animal models, higher local levels of chemokines increase neutrophil recruitment. This has been observed after a challenge with staphylococcal superantigens<sup>35</sup> or injection of recombinant tumor necrosis factor which causes a subsequent increase in local chemokine production.<sup>36</sup> In both of these studies, antibody inhibition of the chemokines reduced local neutrophil recruitment. In a streptococcal arthritis model, high local levels were found relative to systemic levels.<sup>37</sup> Chemokines have also been shown to be important in recruitment of neutrophils into infected urinary bladders.<sup>38</sup> In an interesting model of *Pseudomonas* infection, low doses resulted in only local levels of chemokines but not systemic levels.<sup>39</sup> In humans, increased levels of CXC chemokines in the lung<sup>40</sup> or cerebrospinal fluid<sup>41</sup> correlated with increased numbers of neutrophils.

Our results are similar to a previous study, which documented that the gradient of local to systemic chemokines was closely related to recruitment of neutrophils. For this model, Blackwell and colleagues<sup>42</sup> manipulated the pulmonary chemokine levels by intratracheal injection of endotoxin, whereas systemic levels were altered by intraperitoneal levels of endotoxin. By increasing the plasma levels of the chemokines relative to the pulmonary levels, they were able to dramatically decrease the influx of neutrophils. Our data show a similar pattern in which the ratio of the local to the systemic chemokine concentrations, rather than the absolute local chemokine concentration, dictates the recruitment of neutrophils. Therefore, knowing only the local levels of chemokines, or only the plasma levels, provides only half of the data needed for the equation to predict inflammatory cell recruitment.

## References

1. Clark RA: Activation of the neutrophil respiratory burst oxidase. *J Infect Dis* 1999, 179(Suppl 2):S309–S317
2. Weiss SJ: Tissue destruction by neutrophils [see comments]. *N Engl J Med* 1989, 320:365–376
3. Rollins BJ: Chemokines. *Blood* 1997, 90:909–928

4. Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K: Essential involvement of interleukin-8 (IL-8) in acute inflammation. *J Leukoc Biol* 1994, 56:559–564
5. Van Zee KJ, DeForge LE, Fischer E, Marano MA, Kenney JS, Remick DG, Lowry SF, Moldawer LL: IL-8 in septic shock, endotoxemia, and after IL-1 administration. *J Immunol* 1991, 146:3478–3482
6. Rovai LE, Herschman HR, Smith JB: The murine neutrophil-chemoattractant chemokines LIX, KC, and MIP-2 have distinct induction kinetics, tissue distributions, and tissue-specific sensitivities to glucocorticoid regulation in endotoxemia. *J Leukoc Biol* 1998, 64:494–502
7. Lundblad R, Sandven P, Giercksky KE: The physical nature of a large bowel perforation predicts severity of the subsequent inflammatory response. *Shock* 1995, 3:455–461
8. Holzheimer RG, Schein M, Wittmann DH: Inflammatory response in peritoneal exudate and plasma of patients undergoing planned re-laparotomy for severe secondary peritonitis. *Arch Surg* 1995, 130:1314–1320
9. Walley KR, Lukacs NW, Standiford TJ, Strieter RM, Kunkel SL: Elevated levels of macrophage inflammatory protein 2 in severe murine peritonitis increase neutrophil recruitment and mortality. *Infect Immun* 1997, 65:3847–3851
10. Ebong S, Call D, Nemzek J, Bolgos G, Newcomb D, Remick D: Immunopathologic alterations in murine models of sepsis of increasing severity. *Infect Immun* 1999, 67:6603–6610
11. Baron EJ, Proctor RA: Elicitation of peritoneal polymorphonuclear neutrophils from mice. *J Immunol Methods* 1982, 49:305–313
12. Lentsch AB, Yoshidome H, Cheadle WG, Miller FN, Edwards MJ: Chemokine involvement in hepatic ischemia/reperfusion injury in mice: roles for macrophage inflammatory protein-2 and Kupffer cells [published erratum appears in *Hepatology* Mar;27(3):889 and corrected and republished in *Hepatology* 1998 Apr, 27(4):1172–1177]. *Hepatology* 1998, 27:507–512
13. Espevik T, Nissen-Meyer J: A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J Immunol Methods* 1986, 95:99–105
14. Ebong SJ, Call DR, Bolgos G, Newcomb DE, Granger JL, O'Reilly M, Remick DG: Immunopathologic responses to non-lethal sepsis. *Shock* 1999, 12:118–126
15. Call DR, Remick DG: Low molecular weight heparin is associated with greater cytokine production in a stimulated whole blood model. *Shock* 1998, 10:192–197
16. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM: Production of hybridoma growth factor by human monocytes. *Eur J Immunol* 1987, 17:1411–1416
17. Wollenberg GK, DeForge LE, Bolgos G, Remick DG: Differential expression of tumor necrosis factor and interleukin-6 by peritoneal macrophages in vivo and in culture. *Am J Pathol* 1993, 143:1121–1130
18. Goldblum SE, Wu KM, Jay M: Lung myeloperoxidase as a measure of pulmonary leukostasis in rabbits. *J Appl Physiol* 1985, 59:1978–1985
19. Newcomb D, Bolgos G, Green L, Remick DG: Antibiotic treatment influences outcome in murine sepsis: mediators of increased morbidity. *Shock* 1998, 10:110–117
20. Erzurum SC, Downey GP, Doherty DE, Schwab Bd, Elson EL, Worthen GS: Mechanisms of lipopolysaccharide-induced neutrophil retention. Relative contributions of adhesive and cellular mechanical properties. *J Immunol* 1992, 149:154–162
21. Hintze JL: NCSS 97 Edition. Kaysville, UT, NCSS Statistical Software, 1997
22. Svanborg C, Godaly G, Hedlund M: Cytokine responses during mucosal infections: role in disease pathogenesis and host defence. *Curr Opin Microbiol* 1999, 2:99–105
23. Salkowski CA, Detore G, Franks A, Falk MC, Vogel SN: Pulmonary and hepatic gene expression following cecal ligation and puncture: monophosphoryl lipid A prophylaxis attenuates sepsis-induced cytokine and chemokine expression and neutrophil infiltration. *Infect Immun* 1998, 66:3569–3578
24. Greenberger MJ, Strieter RM, Kunkel SL, Danforth JM, Laichalk LL, McGillicuddy DC, Standiford TJ: Neutralization of macrophage inflammatory protein-2 attenuates neutrophil recruitment and bacterial clearance in murine *Klebsiella pneumoniae*. *J Infect Dis* 1996, 173:159–165
25. Tsai WC, Strieter RM, Wilkowski JM, Bucknell KA, Burdick MD, Lira SA, Standiford TJ: Lung-specific transgenic expression of KC en-

- hances resistance to *Klebsiella pneumoniae* in mice. *J Immunol* 1998, 161:2435–2440
26. Mulligan MS, Jones ML, Bolanowski MA, Baganoff MP, Deppeler CL, Meyers DM, Ryan US, Ward PA: Inhibition of lung inflammatory reactions in rats by an anti-human IL-8 antibody. *J Immunol* 1993, 150: 5585–5595
  27. Lira SA, Zalamea P, Heinrich JN, Fuentes ME, Carrasco D, Lewin AC, Barton DS, Durham S, Bravo R: Expression of the chemokine N51/KC in the thymus and epidermis of transgenic mice results in marked infiltration of a single class of inflammatory cells. *J Exp Med* 1994, 180:2039–2048
  28. Bozic CR, Kolakowski Jr LF, Gerard NP, Garcia-Rodriguez C, von Uexkull-Guldenband C, Conklyn MJ, Breslow R, Showell HJ, Gerard C: Expression and biologic characterization of the murine chemokine KC. *J Immunol* 1995, 154:6048–6057
  29. Jerva LF, Sullivan G, Lolis E: Functional and receptor binding characterization of recombinant murine macrophage inflammatory protein 2: sequence analysis and mutagenesis identify receptor binding epitopes. *Protein Sci* 1997, 6:1643–1652
  30. Bozic CR, Gerard NP, von Uexkull-Guldenband C, Kolakowski LFJ, Conklyn MJ, Breslow R, Showell HJ, Gerard C: The murine interleukin 8 type B receptor homologue and its ligands. Expression and biological characterization. *J Biol Chem* 1994, 269:29355–29358
  31. Gimbrone Jr MA, Obin MS, Brock AF, Luis EA, Hass PE, Hebert CA, Yip YK, Leung DW, Lowe DG, Kohr WJ, Darbonne WC, Bechtol KB, Baker JB: Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. *Science* 1989, 246:1601–1603
  32. Hechtman DH, Cybulsky MI, Fuchs HJ, Baker JB, Gimbrone Jr MA: Intravascular IL-8. Inhibitor of polymorphonuclear leukocyte accumulation at sites of acute inflammation. *J Immunol* 1991, 147:883–892
  33. Ley K, Baker JB, Cybulsky MI, Gimbrone Jr MA, Luscinskas FW: Intravenous interleukin-8 inhibits granulocyte emigration from rabbit mesenteric venules without altering L-selectin expression or leukocyte rolling. *J Immunol* 1993, 151:6347–6357
  34. Simonet WS, Hughes TM, Nguyen HQ, Trebasky LD, Danilenko DM, Medlock ES: Long-term impaired neutrophil migration in mice over-expressing human interleukin-8. *J Clin Invest* 1994, 94:1310–1319
  35. Tessier PA, Naccache PH, Diener KR, Gladue RP, Neote KS, Clark-Lewis I, McColl SR: Induction of acute inflammation in vivo by staphylococcal superantigens. II. Critical role for chemokines, ICAM-1, and TNF-alpha. *J Immunol* 1998, 161:1204–1211
  36. Tessier PA, Naccache PH, Clark-Lewis I, Gladue RP, Neote KS, McColl SR: Chemokine networks in vivo: involvement of C-X-C and C-C chemokines in neutrophil extravasation in vivo in response to TNF-alpha. *J Immunol* 1997, 159:3595–3602
  37. Tissi L, Puliti M, Barluzzi R, Orefici G, von Hunolstein C, Bistoni F: Role of tumor necrosis factor alpha, interleukin-1beta, and interleukin-6 in a mouse model of group B streptococcal arthritis. *Infect Immun* 1999, 67:4545–4550
  38. Hangen DH, Segall GM, Harney EW, Stevens JH, McDougall IR, Raffin TA: Kinetics of leukocyte sequestration in the lungs of acutely septic primates: a study using 111In-labeled autologous leukocytes. *J Surg Res* 1990, 48:196–203
  39. Terashima T, Matsubara H, Nakamura M, Sakamaki F, Waki Y, Soejima K, Tasaka S, Nakamura H, Sayama K, Ishizaka A, Kanazawa M: Local *Pseudomonas* instillation induces contralateral lung injury and plasma cytokines. *Am J Respir Crit Care Med* 1996, 153:1600–1605
  40. Bellocq A, Antoine M, Flahault A, Philippe C, Crestani B, Bernaudin JF, Mayaud C, Milleron B, Baud L, Cadranet J: Neutrophil alveolitis in bronchioloalveolar carcinoma: induction by tumor-derived interleukin-8 and relation to clinical outcome. *Am J Pathol* 1998, 152:83–92
  41. Sprenger H, Rosler A, Tonn P, Braune HJ, Huffmann G, Gemsa D: Chemokines in the cerebrospinal fluid of patients with meningitis. *Clin Immunol Immunopathol* 1996, 80:155–161
  42. Blackwell TS, Lancaster LH, Blackwell TR, Venkatakrishnan A, Christman JW: Chemotactic gradients predict neutrophilic alveolitis in endotoxin-treated rats. *Am J Respir Crit Care Med* 1999, 159:1644–1652