Gamma Interferon Prevents the Inhibitory Effects of Oxidative Stress on Host Responses to Escherichia coli Infection

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Oxidative stress occurs in animals challenged with bacterial endotoxin and can affect the expression of important host inflammatory genes. However, much less is known about the effects of oxidative stress on responses to gram-negative bacteria. The current study compared the effects of redox imbalance on hepatic responses of mice to Escherichia coli bacteria versus purified endotoxic lipopolysaccharide (LPS). Oxidative stress induced by glutathione depletion virtually eliminated hepatic tumor necrosis factor alpha responses to both E. coli and LPS. Inducible NO synthase (iNOS) and intercellular adhesion molecule-1 (ICAM-1) expression was also markedly inhibited by glutathione depletion in LPS-challenged mice, but was unaffected in E. coli-infected animals. Three findings suggested that gamma interferon (IFN-γ) production explained the differences between LPS and bacterial challenge. Glutathione depletion completely inhibited the IFN-γ response to LPS, but only partially inhibited IFN-γ production in infected mice. Exogenous IFN-γ restored iNOS and ICAM-1 responses to LPS in stressed mice. Conversely, IFN-γ-deficient, glutathione-depleted mice showed a marked decrease in iNOS and ICAM-1 expression when challenged with E. coli. These findings indicate that both the nature of the microbial challenge and the production of IFN-γ can be important in determining the effects of redox imbalance during gram-negative bacterial infections.

Sepsis is a response to infection characterized by the production of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and gamma interferon (IFN-γ), and the release of highly reactive oxygen and nitrogen intermediates. These oxidizing species are thought to contribute to much of the end-stage tissue damage seen in this disease. Considerable evidence indicates that oxygen and nitrogen metabolites can also regulate the expression of genes that are induced by bacterial components in vitro (7, 16, 20, 35, 38) by causing oxidative or reductive/oxidative (redox) stress within cells (7, 16, 20, 35). Similarly, Nathens et al. (29) showed that the depletion of the antioxidant glutathione in vivo inhibited the expression of intercellular adhesion molecule 1 (ICAM-1) in the lungs of rats challenged with bacterial lipopolysaccharide (LPS). Significant tissue redox imbalance occurs in both endotoxia and gram-negative bacterial infections (32, 36, 43), suggesting that oxidative stress may be an important regulator of inflammatory mediator production during sepsis. Consistent with this prediction, Matsushak and his colleagues (28, 47) reported that oxidative stress resulting from hypoxia-reoxygenation of perfused rat livers inhibited the in vivo production of TNF-α and IL-1β in response to challenge with live Escherichia coli bacteria.

Glutathione is the most plentiful nonprotein thiol found in most cells and as such serves as a major scavenger of intracellular oxidants (19). Both the depletion of total intracellular glutathione and the oxidation of the glutathione sulfhydryl (GSH) to form glutathione disulfide significantly alters the redox state of cells. For example, oxidative stress occurs when cells are exposed to electrophiles, such as diethyl maleate (DEM), that conjugate directly with GSH and cause the export of the resulting glutathione adduct from the cell (19). Likewise, buthionine sulfoximine (BSO) depletes GSH in vitro and in vivo by specifically inhibiting γ-glutamylcysteine synthase, a rate-limiting enzyme in GSH biosynthesis (18). In turn, glutathione redox imbalance can lead to the expression of a number of “stress-responsive” genes, including those coding for heat shock proteins (15).

Animal models in which endotoxic LPS has served as a surrogate for infection have proven valuable for defining the basic pathophysiological responses to gram-negative bacteria and the important inflammatory cells and soluble mediators of sepsis. However, animal endotoxicosis models have not always predicted the nature of host inflammatory responses to gram-negative bacterial challenge (6, 8, 12, 21, 48). For this reason, we have compared the effects of tissue oxidative stress on the expression of several inflammatory genes in LPS-challenged and E. coli-infected mice. The mouse liver was chosen for this purpose because many important inflammatory mediators, including TNF-α, IFN-γ, CD14, inducible NO synthase (iNOS), and ICAM-1, are expressed by hepatic cells following either LPS or bacterial challenge. The organ also suffers substantial damage in sepsis that is mediated, in part, by the expression of these genes (23, 33, 41). Importantly, oxidative stress occurs in the liver when mice are given glutathione-depleting agents (46).

IFN-γ is an important mediator of endotoxemia and gram-negative bacterial sepsis in a number of mammalian species. The cytokine enhances LPS lethality in rodents, and antibody to IFN-γ can diminish LPS-induced inflammatory responses and lethality (2, 22, 25, 40). Likewise, the targeted disruption of the genes for either IFN-γ or its receptor subunits has been

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shown to modify responses to LPS, including lethality in several rodent models (3, 24, 37). Kamijo et al. (24) showed that IFN-γ mediated the ability of Corynebacterium parvum to enhance LPS-induced cytokine production, iNOS expression, and lethality in mice. Salkowski et al. (37) used IFN-γ-null mice to show that the sustained induction of mouse hepatic iNOS mRNA by LPS required IFN-γ gene expression. Similar conclusions regarding the importance of IFN-γ have been made with many but not all gram-negative infection models (13, 26, 27). Kohler et al. (26) reported that IFN-γ enhanced lethality in mice challenged with E. coli bacteria and that neutralizing antibody to the cytokine improved survival. Despite these findings, very little is known about the effects of oxidative stress on the production of IFN-γ by animals challenged with gram-negative bacteria or bacterial LPS.

Because mice that have been injected with LPS or infected with E. coli bacteria show significantly decreased levels of hepatic glutathione as early as 6 h postchallenge (M. J. Parmely, F. Wang, and D. Wright, unpublished data), we have asked to what extent tissue redox imbalance alters hepatic responses to LPS or infection. The present study will show that glutathione depletion inhibits LPS-induced iNOS and ICAM-1 expression in the mouse liver but has no apparent effect on these responses in E. coli-infected mice. The ability of glutathione-depleted, infected mice to produce IFN-γ appears to prevent many of the inhibitory effects of oxidative stress on these responses, suggesting that IFN-γ-dependent signaling pathways are utilized during infection that are not activated following LPS challenge. These findings predict that individuals in redox imbalance, including patients whose glutathione levels are intentionally manipulated to enhance the action of certain cancer chemotherapeutic agents (1), have the potential to show substantially altered responses to bacterial challenge.

MATERIALS AND METHODS

Reagents. E. coli O111:B4 LPS, DEM, and BSO were purchased from Sigma Chemical Co. (St. Louis, Mo.). Recombinant mouse IFN-γ was provided by Genentech (South San Francisco, Calif.). The following antibodies were used in the present study: rat anti-mouse TNF-α (MP6-XT22; PharMingen, San Diego, Calif.), rabbit anti-mouse iNOS (SC-650; Santa Cruz Biotechnology, Santa Cruz, Calif.), goat anti-mouse ICAM-1 (SC-1511; Santa Cruz), rat anti-mouse macrophage F4/80 (a gift of Joan Hunt, University of Kansas Medical Center), rat anti-mouse CD14 (rmC5-3; PharMingen), rabbit anti-rat heat shock protein-32 (HSP-32) (SPA-895; StressGen, Victoria, BC, Canada), biotinylated goat anti-phage F4/80 (a gift of Joan Hunt, University of Kansas Medical Center). Wild-type BALB/c (IFN-γ1/2−/2−), 1C). Thus, the treatment of mice with DEM or BSO caused significant redox imbalance in their livers.

Measurement of serum TNF-α and IFN-γ. Serum TNF levels were measured by the L929 cell bioassay as previously described (34). Serum IFN-γ concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (PharMingen). Because the samples were first diluted, the detection limits vary with the assay and are specified in each case.

Statistical analysis. Each experiment was performed at least twice, and representative results are shown. Student’s two-tailed t test was used to determine statistical significance (P < 0.01). Serum cytokine concentrations that were undetectable were assigned the value of the detection limit of the assay for the purposes of calculating group means.

RESULTS

Glutathione depletion and oxidative stress responses in the mouse liver. A single i.p. injection of DEM (5.3 mmol/kg) depleted hepatic glutathione levels in CF1 mice by greater than 90%, a condition which persisted for several hours (Fig. 1A). This effect was accompanied by the elevated expression of HSP-32, an oxidative stress-responsive gene, by liver Kupffer cells, hepatocytes, and hepatic sinusoidal endothelial cells (Fig. 1B and 2A). Similarly, injecting mice twice with BSO (5 mmol/kg) significantly decreased the glutathione levels in their livers and induced a comparable pattern of HSP-32 expression (Fig. 1C). Thus, the treatment of mice with DEM or BSO caused significant redox imbalance in their livers.

Effects of glutathione depletion on host responses to live E. coli bacteria. To determine the effects of oxidative stress on...
hepatic inflammatory responses to E. coli, groups of mice were treated with either vehicle (sesame oil) or DEM and challenged i.p. with $4 \times 10^7$ to $5 \times 10^7$ CFU of E. coli O111:B4 bacteria 2 h later. This dose represents 3 to 4 50% lethal doses for this bacterial strain when injected by the i.p. route. Sera were collected 90 min later, and circulating TNF-α concentrations were measured. While mice that had been treated with vehicle and challenged with bacteria showed high circulating levels of TNF-α, DEM treatment almost completely blocked this response to bacterial challenge (Fig. 3A). This inhibitory effect was due to a marked decrease in TNF-α synthesis, as determined by enumerating TNF-α-producing Kupffer cells in the liver by immunohistology (Fig. 2B and C).

The effects of glutathione depletion on macrophage activation in the liver were selective in the sense that E. coli-induced Kupffer cell iNOS expression was unaffected by DEM treatment (Fig. 2D and E). Hepatocytes and sinusoidal endothelial cells also expressed iNOS 6 h after an i.p. challenge with E. coli, but neither of these responses was affected by DEM treatment. Similarly, the induction of ICAM-1 expression by endothelial cells and Kupffer cells was comparable in the vehicle and DEM-treated groups (Fig. 2F and G). Thus, unlike the TNF-α response in the liver, hepatic iNOS and ICAM-1 responses to bacterial infection were unaffected by glutathione depletion. Importantly, depleting hepatic glutathione with BSO produced an identical pattern of responses to E. coli challenge (Table 1).

Responses to E. coli bacteria versus purified E. coli LPS. The effects of DEM on the TNF-α, iNOS, ICAM-1, and CD14 responses to E. coli O111:B4 bacteria were then compared to its effects on responses to chemically purified LPS of the same E. coli serotype. For this purpose, mice were injected i.p. with 100 μg of LPS, a dose which elicited inflammatory responses comparable to those elicited by live bacterial challenge. The semiquantitative data that are summarized in Fig. 4 were derived from immunohistology experiments of the type shown in Fig. 2, in which the densities or staining intensities of positive cells were recorded. It is clear from this analysis that Kupffer cell TNF-α responses to both E. coli and LPS challenge were significantly inhibited by DEM treatment, and this effect was confirmed when serum TNF-α responses to bacterial and LPS challenge were measured (Fig. 3A and B). Although all other responses to E. coli challenge were unaffected by DEM treatment (Fig. 4, left panels), the effects of glutathione depletion on hepatic responses to LPS were entirely different. Kupffer cell ICAM-1, hepatocyte, and endothelial cell iNOS and endothelial cell ICAM-1 responses to LPS were all markedly inhibited by DEM (Fig. 4, right panels). Thus, the ability of redox imbalance to regulate hepatic inflammatory responses to live gram-negative bacteria was fundamentally different from its effects on the same responses to LPS.

IFN-γ prevents the inhibitory effects of glutathione depletion in the liver. We next asked whether the production of IFN-γ was inhibited by glutathione depletion and whether the ability of the different groups of mice to produce IFN-γ might explain the findings described above. Control and DEM-treated CF1 mice were challenged with either LPS or live bacteria, and 6 h later their sera were collected for the measurement of IFN-γ. LPS and E. coli organisms stimulated comparable IFN-γ responses (Fig. 5). Of interest, DEM-treated
mice did not produce detectable IFN-γ when challenged with LPS. However, when challenged with bacteria, the serum IFN-γ levels of DEM-treated mice, although decreased, were substantial (i.e., at least eight times those of unchallenged mice). This effect was also seen in mice of the C57BL/6 and BALB/c strains and indicates that infected animals maintained the ability to produce significant quantities of IFN-γ despite glutathione depletion.

To ascertain whether IFN-γ produced in response to infection provided an alternative signal for iNOS and ICAM-1 expression in DEM-treated mice that was not induced by LPS, animals were challenged with either 100 μg of LPS or LPS plus 2 μg of mouse recombinant IFN-γ. This dose of IFN-γ by itself did not induce iNOS or ICAM-1 expression, but has been shown to induce other inflammatory responses to LPS (22, 25, 26). Six hours after challenge, liver samples were obtained, and hepatic iNOS and ICAM-1 responses were measured. The results, shown in Fig. 2H to K and summarized in Table 2, indicate that exogenous IFN-γ significantly altered the host responses in the livers of glutathione-depleted, LPS-challenged mice. While the TNF-α response was not restored by IFN-γ, hepatic iNOS and ICAM-1 expression was significantly enhanced when IFN-γ was coinjected with LPS. Thus, the combination of LPS and IFN-γ induced the same responses in DEM-treated mice (i.e., hepatocyte iNOS [Fig. 2I] and Kupffer cell and endothelial cell ICAM-1 [Fig. 2K]) that were seen after E. coli challenge. Recombinant IFN-γ did not increase the frequency of iNOS-positive Kupffer cells in DEM-treated, LPS-stimulated mice (Table 2), indicating that the cytokine did not simply prime cells for all LPS-induced responses. Because the frequency of F4/80-positive Kupffer cells was equivalent in the two treatment groups, it is also unlikely that IFN-γ increased Kupffer cell ICAM-1 expression by recruiting mononuclear phagocytes to the liver. Rather, the results indicate that exogenous IFN-γ converted several stress-sensitive, LPS-induced responses to stress-resistant responses that mimicked what was seen in infected animals.

To determine whether IFN-γ mediated these inflammatory responses to E. coli challenge in glutathione-depleted mice, wild-type BALB/c (IFN-γ+/+) and homozygous IFN-γ-null (IFN-γ−/−) mutant mice were treated with either vehicle or DEM and then challenged with E. coli bacteria. Glutathione-depleted wild-type mice retained the ability to express both hepatic ICAM-1 and iNOS when challenged with bacteria (Fig. 2L and M and Table 3), despite a significant decrease in their IFN-γ responses. In contrast, IFN-γ-null mice that had been treated with DEM showed markedly reduced hepatic iNOS and ICAM-1 expression (i.e., hepatocyte iNOS, Kupffer cell ICAM-1, and sinusoidal endothelial cell ICAM-1 and ICAM-1) (Fig. 2N and Table 3). This was also seen in IFN-γ-deficient mice that had been treated with vehicle and then challenged with bacteria. Therefore, the production of IFN-γ was essential for these responses in infected animals. Numerous ICAM-1-positive leukocytes were present within the hepatic sinusoids of IFN-γ-null mice 6 h after infection (Fig. 2N), but these intrasinusoidal leukocytes did not express the F4/80 monocyte-macrophage marker (Fig. 2O).

**DISCUSSION**

Previously we reported that hepatic TNF-α and iNOS responses to *Salmonella enteritidis* LPS were selectively inhibited

![FIG. 2. Glutathione depletion differentially inhibits hepatic inflammatory responses in mice challenged with 5 × 10⁷ E. coli bacteria. All injections were by the i.p. route. Immunohistochemistry was used to detect the expression of specific proteins in the livers of the following groups of mice. (A) HSP-32 staining in mice 5 h after the injection of DEM (see Fig. 1A). Note the expression of HSP-32 by Kupffer cells (solid arrow), hepatocytes (open arrow) and hepatic sinusoidal endothelial cells (arrowhead). (B) TNF-α staining in Kupffer cells (solid arrow) 1.5 h after vehicle-treated mice were challenged with *E. coli* bacteria. (C) TNF-α staining 1.5 h after DEM-treated mice were challenged with *E. coli*. Note the absence of TNF-α expression. (D) iNOS staining in Kupffer cells (solid arrow), hepatocytes (open arrow), and hepatic sinusoidal endothelial cells (arrowhead) 6 h after challenging vehicle-treated mice with *E. coli*. (E) iNOS staining in DEM-treated mice challenged with *E. coli*. (F) ICAM-1 staining of Kupffer cells (solid arrow) and endothelial cells (arrowhead) 6 h after challenge of the vehicle control group with *E. coli*. (G) ICAM-1 staining in DEM-treated, *E. coli*-challenged mice. (H) iNOS staining in DEM-treated mice that were challenged with 4 mg of LPS/kg of body weight showing positive Kupffer cells (solid arrow) and sinusoidal endothelial cells (arrowhead). (I) iNOS staining in DEM-treated mice that were challenged with 4 mg LPS plus 80 μg of recombinant IFN-γ per kg of body weight. Note the staining of all three hepatic cell types, which is similar to that seen in infected mice (panel E above). (J) ICAM-1 staining in DEM-treated mice that were challenged with LPS. (K) ICAM-1 staining in DEM-treated mice challenged with LPS and recombinant IFN-γ. The intense staining is similar to that seen in infected mice (panel G above). (L) ICAM-1 staining of Kupffer cells (solid arrow) and endothelial cells (arrowhead) in wild-type BALB/c mice infected with *E. coli*. (M) ICAM-1 staining in wild-type BALB/c mice treated with DEM and then infected with *E. coli*. Note the similar staining pattern as in panel L. (N) ICAM-1 staining in IFN-γ-null mice treated with DEM and challenged with *E. coli*. Note the presence of sinusoidal leukocytes expressing ICAM-1 (circled). (O) F4/80 staining of Kupffer cells (solid arrow) in IFN-γ-null mice treated with DEM and challenged with *E. coli*. Sinusoidal leukocytes (circled) are negative. Original magnification, ×250.**
in C3HeB/FeJ mice by glutathione depletion (46). The present study sought to extend these findings to mice with gram-negative bacterial infections and has yielded a number of unexpected findings regarding the effects of tissue redox imbalance. We selected a monomicrobial gram-negative infection model to enable a direct comparison between bacterial infection and challenge with LPS purified from the same species and serotype (i.e., *E. coli* O111:B4). Although the magnitude and cell type-specific expression of several different inflammatory mediators were comparable in LPS-challenged and *E. coli*-infected mice (Fig. 3, 4, and 5), the effects of oxidative stress on responses to these two microbial stimuli were fundamentally different. Glutathione depletion significantly inhibited hepatic TNF-α, iNOS, and ICAM-1 responses to LPS and eliminated IFN-γ production. By contrast, only one of these responses (TNF-α production) was inhibited to a similar extent in mice that were challenged with live *E. coli* bacteria. The fact that DEM and BSO treatment produced essentially identical inhibitory effects supports the conclusion that glutathione depletion per se was responsible for these changes. Because hepatocytes, Kupffer cells, and sinusoidal endothelial cells each expressed HSP-32 following DEM and BSO treatment and showed specific changes in their responses to LPS challenge, it is likely that glutathione depletion...
that glutathione depletion directly affected each of these cell types.

We cannot presently exclude the possibility that the differences between the effects of oxidative stress on host responses to LPS versus live bacteria resulted from differences in the tissue distribution, physical-chemical composition, and/or dose of endotoxins delivered under these two conditions of microbial challenge. Indeed, Ge et al. (17) have reported that LPS localizes to different hepatic cell types in rats injected with chemically purified LPS versus animals infected with live E. coli bacteria. We have recently reproduced these findings in C3H mice (M. J. Parmely and F. Wang, unpublished data). In LPS-challenged animals, immunoreactive LPS was found in both hepatocytes and Kupffer cells, whereas antigenic LPS was not detected in hepatocytes following E. coli challenge. Kupffer cells accumulated LPS under both conditions. In the present study, LPS-induced but not E. coli-induced Kupffer cell ICAM-1 expression was inhibited by oxidative stress. Thus, glutathione depletion had remarkably different effects on the Kupffer cell ICAM-1 responses to LPS and E. coli, despite similar LPS localization to this cell type following these two forms of microbial challenge.

Responsiveness to LPS is not necessary for the induction of many host responses to viable E. coli bacteria (5, 14), indicating that non-LPS bacterial components (e.g., bacterial DNA) also activate inflammatory mediator expression in infected animals. If these responses are ultimately shown to arise from novel signaling pathways that are activated in infected but not endotoxemic mice, the present study will have established that at least some LPS-independent pathways are relatively resistant to changes in cellular redox state.

The expression of ICAM-1 and iNOS in the rodent liver is induced by endotoxin (10, 37, 45) and ischemia-reperfusion (4), and TNF-α appears to provide an important proximal signal for the induction of these genes (4, 11, 37). For this reason, we were not surprised that LPS-induced hepatic ICAM-1 and iNOS expression declined in parallel with the loss of TNF-α production in glutathione-depleted, LPS-challenged mice. However, the hepatic ICAM-1 and iNOS responses to E. coli were undiminished by DEM or BSO treatment, despite a comparable inhibition of TNF-α production in infected animals. These findings strongly suggest that ICAM-1 and iNOS induction during gram-negative bacterial infections is not entirely dependent upon TNF-α production and that alternative signaling pathways exist for eliciting these responses.

A number of findings reported here suggest that the production of IFN-γ is important in determining the ultimate effects of redox imbalance on hepatic inflammatory responses to gram-negative bacteria in the mouse. Whereas serum IFN-γ was undetectable in glutathione-depleted mice that had been challenged with LPS, DEM treatment failed to completely block IFN-γ responses to bacterial challenge. When IFN-γ was coadministered with LPS to DEM-treated animals, their hepatic inflammatory responses were indistinguishable from those of glutathione-depleted, E. coli-infected mice. In other words, hepatic iNOS and ICAM-1 responses to LPS were completely restored by coincubation with exogenous IFN-γ. Conversely, the ability of glutathione-depleted mice to produce IFN-γ was essential for hepatic iNOS and ICAM-1 responses to E. coli challenge, because the responses were absent from DEM-treated IFN-γ-deficient mice. The finding that infected IFN-γ-deficient mice with normal hepatic glutathione also failed to express iNOS or ICAM-1 strongly suggests that DEM acts by inhibiting IFN-γ production rather than its action. Overall, these results indicate that IFN-γ is an important signal for the induction of hepatic iNOS and ICAM-1 responses to gram-negative bacterial infection during oxidative stress. Conversely, the lack of iNOS and ICAM-1 responses in glutathione-depleted, LPS-challenged mice appears to result from their failure to produce this cytokine.

The production of IFN-γ in mice challenged with LPS is mediated almost entirely by activated NK-1<sup>a</sup> natural killer cells and NK T cells (9, 30, 31, 39, 42). Non-LPS microbial components often induce more complex patterns of cell acti-

### TABLE 2. IFN-γ restores the hepatic iNOS and ICAM-1 responses to LPS in DEM-treated CF1 mice<sup>a</sup>

<table>
<thead>
<tr>
<th>Antigen (cell type)</th>
<th>Treatment</th>
<th>Serum IFN-γ (pg/ml)</th>
<th>Positive cells&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Intensity score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hep</td>
<td>EC</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(cells/HPF)</td>
<td>(cells/HPF)</td>
</tr>
<tr>
<td>TNF-α LPS</td>
<td>Vehicle</td>
<td>3,360</td>
<td>38.4 ± 9.9</td>
<td>20.3 ± 3.2</td>
</tr>
<tr>
<td>TNF-α LPS</td>
<td>DEM</td>
<td>33.6 ± 9.8</td>
<td>18.7 ± 5.5</td>
<td>23.3 ± 7.4</td>
</tr>
<tr>
<td>iNOS LPS</td>
<td>Vehicle</td>
<td>275</td>
<td>33.6 ± 9.8</td>
<td>18.7 ± 5.5</td>
</tr>
<tr>
<td>iNOS LPS</td>
<td>DEM</td>
<td>33.6 ± 9.8</td>
<td>18.7 ± 5.5</td>
<td>23.3 ± 7.4</td>
</tr>
<tr>
<td>ICAM-1 LPS</td>
<td>Vehicle</td>
<td>&lt;3 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.4 ± 1.0</td>
</tr>
<tr>
<td>ICAM-1 LPS</td>
<td>DEM</td>
<td>33.6 ± 9.8</td>
<td>18.7 ± 5.5</td>
<td>23.3 ± 7.4</td>
</tr>
</tbody>
</table>

* BALB/c wild-type or mutant IFN-γ-null mice (five per group) were treated with vehicle or DEM and challenged with E. coli 2 h later. Liver tissue and blood samples were recovered 6 h later. Shown are the average values from two experiments. The detection limit of the IFN-γ assay was 33 pg/ml. *<sup>a</sup> this group differed significantly from the corresponding wild-type group (P < 0.01). Also see Table 1, footnote a.

* Values are means ± SD.

### TABLE 3. Role of IFN-γ in regulating E. coli-induced hepatic inflammatory mediator expression in DEM-treated mice<sup>b</sup>

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mouse strain</th>
<th>Serum IFN-γ (pg/ml)</th>
<th>Antigen (cell type)-positive cells/HPF&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Intensity score</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hep</td>
<td>KC</td>
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<tr>
<td>Vehicle IFN-γ&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3,360</td>
<td>38.4 ± 9.9</td>
<td>20.3 ± 3.2</td>
</tr>
<tr>
<td>DEM IFN-γ&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>275</td>
<td>33.6 ± 9.8</td>
<td>18.7 ± 5.5</td>
</tr>
<tr>
<td>Vehicle IFN-γ&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>&lt;33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.1 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.4 ± 1.0</td>
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<tr>
<td>DEM IFN-γ&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>&lt;33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.7 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.4 ± 3.4</td>
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* BALB/c wild-type or mutant IFN-γ-null mice (five per group) were treated with vehicle or DEM and challenged with E. coli 2 h later. Liver tissue and blood samples were recovered 6 h later. Shown are the average values from two experiments. The detection limit of the IFN-γ assay was 33 pg/ml. *<sup>a</sup> this group differed significantly from the corresponding wild-type group (P < 0.01). Also see Table 1, footnote a.

* Values are means ± SD.

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<sup>a</sup> CF1 mice (five per group) were injected i.p. with DEM (5.3 mmol/kg) 2 h prior to challenge. Mice were challenged i.p. with either 100 μg of E. coli O111:B4 LPS or LPS plus 2 μg of recombinant IFN-γ. Liver samples were recovered 6 h later for immunohistology and read at ×400 magnification.

<sup>b</sup> See Table 1, footnote a and b. * these groups differed significantly from their controls (LPS only) (P < 0.01).
viation and IFN-γ production (9, 30, 39). For example, Nguyen and Biron (30) showed that C57BL/6 mice infected with lymphocytic choriomeningitis virus produced greatly elevated levels of circulating IFN-γ when challenged with LPS compared to uninfected, LPS-challenged mice. Mice challenged only with virus produced very little IFN-γ. The heightened response to coinfection was due to the production of IFN-γ by CD4+ and CD8+ T cells in addition to NK cells. Likewise, Seki et al. (39) have reported that polymicrobial peritonitis in BALB/c and C57BL/6 mice resulting from cecal ligation and puncture activated large numbers of IFN-γ-producing cells in the liver. Approximately 20% of these cells lacked the NK-1 marker.

These reports raise the interesting possibility that undefined components of gram-negative bacteria induce novel IFN-γ responses by cells not activated in LPS-challenged animals. The present study would predict that this latter portion of the IFN-γ response to infection is not inhibited by tissue redox imbalance and mediates important host responses to infection.

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