Regulation of Macrophage Chemokine Expression by Lipopolysaccharide In Vitro and In Vivo

Karen M. Kopydlowski, Cindy A. Salkowski, M. Joshua Cody, Nico van Rooijen, Jennifer Major, Thomas A. Hamilton and Stefanie N. Vogel

*J Immunol* 1999; 163:1537-1544; ;
http://www.jimmunol.org/content/163/3/1537

References
This article cites 66 articles, 42 of which you can access for free at:
http://www.jimmunol.org/content/163/3/1537.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Regulation of Macrophage Chemokine Expression by Lipopolysaccharide In Vitro and In Vivo

Karen M. Kopydlowski, Cindy A. Salkowski, M. Joshua Cody, Nico van Rooijen, Jennifer Major, Thomas A. Hamilton, and Stefanie N. Vogel

The host response to Gram-negative LPS is characterized by an influx of inflammatory cells into host tissues, which is mediated, in part, by localized production of chemokines. The expression and function of chemokines in vivo appears to be highly selective, though the molecular mechanisms responsible are not well understood. All CXC (IFN-γ-inducible protein (IP)-10, macrophage inflammatory protein (MIP)-2, and KC) and CC (JE/monocyte chemoattractant protein (MCP)-1, MCP-5, MIP-1α, MIP-1β, and RANTES) chemokine genes evaluated were sensitive to stimulation by LPS in vitro and in vivo. While IL-10 suppressed the expression of all LPS-induced chemokine genes evaluated in vitro, treatment with IFN-γ selectively induced IP-10 and MCP-5 mRNAs, but inhibited LPS-induced MIP-2, KC, JE/MCP-1, MIP-1α, and MIP-1β mRNA and/or protein. Like the response to IFN-γ, LPS-mediated induction of IP-10 and MCP-5 was Stat1 dependent. Interestingly, only the IFN-γ-mediated suppression of LPS-induced KC gene expression was IFN regulatory factor-2 dependent. Treatment of mice with LPS in vivo also induced high levels of chemokine mRNA in the liver and lung, with a concomitant increase in circulating protein. Hepatic expression of MIP-1α, MIP-1β, RANTES, and MCP-5 mRNAs were dramatically reduced in Kupffer cell-depleted mice, while IP-10, KC, MCP-2, and MCP-1 were unaffected or enhanced. These findings indicate that selective regulation of chemokine expression in vivo may result from differential response of macrophages to pro- and antiinflammatory stimuli and to cell type-specific patterns of stimulus sensitivity. Moreover, the data suggest that individual chemokine genes are differentially regulated in response to LPS, suggesting unique roles during the sepsis cascade. The Journal of Immunology, 1999, 163: 1537–1544.

Among the cytokine products expressed by LPS-stimulated macrophages are chemoattractant cytokines or chemokines. These products are believed to control the nature and magnitude of inflammatory cell infiltration (7–9). The chemokine superfamily consists of small (7–14 kDa), basic, heparin-binding proteins that are categorized into four subfamilies, CXC, CC, C (10), and CX3C, consisting of small (7–14 kDa), basic, heparin-binding proteins that are categorized into four subfamilies, CXC, CC, C (10), and CX3C. The CXC chemokines, IFN-γ-inducible protein (IP)-10, had been reported to recruit monocytes, T lymphocytes, and NK cells (16, 17); however, the recently cloned IP-10 receptor, CXCR3, has only been demonstrated on NK cells and activated T lymphocytes (18). The CC chemokines, JE/monocyte chemoattractant protein (MCP)-1, MCP-5, MIP-1α, MIP-1β, and RANTES are all chemotactic for monocytes/macrophages and T cells (19), although several CC chemokines have been reported to attract NK cells, basophils, eosinophils, dendritic cells, and mast cells (7, 8). Examination of chemokine expression in vivo has revealed a much more restricted pattern of expression than would be predicted from analysis of chemokine expression in vitro. Furthermore, though many different chemokines have been detected at sites of inflammation and in tissues during systemic sepsis...
(20–25), numerous reports demonstrate nonredundant roles for individual chemokines. For example, anti-MIP-2 Ab administration reduced neutrophil infiltration into the lung, but failed to protect against LPS-induced mortality (23), while neutralization of MIP-2 in a murine model of peritonitis decreased both mortality and neutrophil recruitment into the peritoneal cavity (21). Increased pulmonary expression of MIP-1α and RANTES has been associated with the influx of monocytes into the lungs of mice after LPS challenge (22, 24). Administration of anti-MIP-1α Ab to mice reduced monocyte, lymphocyte, and neutrophil infiltration into the lungs with only a modest effect on mortality (24), while anti-RANTES Ab only reduced monocyte influx into the lungs (22). In contrast, JE/MCP-1 protected mice from LPS-induced mortality (20).

While the majority of recent studies have focused on the role of individual chemokines in sepsis or endotoxemia, we sought to enhance our understanding of the complex inflammatory cascade initiated after LPS challenge by systematically evaluating a more comprehensive panel of CXC and CC chemokine genes. To this end, the regulation of LPS-induced mRNA expression of the CXC chemokines, IP-10, MIP-2, and KC, as well as the CC chemokines, JE/MCP-1, MCP-5, MIP-1α, MIP-1β, and RANTES, was evaluated both in vitro and in vivo. We wished to evaluate the cellular and molecular mechanisms that may be responsible for selective expression and function in vivo. Despite apparent functional redundancies and intrafamily structural homologies, these studies clearly demonstrate that individual chemokine genes are differentially regulated. Novel key findings from this study include: 1) IL-10 down-regulates LPS-induced mRNA expression for all chemokine genes examined, 2) IFN-γ induces or inhibits distinct subsets of LPS-induced chemokine genes, 3) only induction of IP-10 and MCP-5 mRNA by LPS or IFN-γ is Stat1 dependent, 4) only IFN-γ-mediated inhibition of KC expression was dependent upon IFN regulatory factor (IRF)-2 (but not IRF-1), and 5) hepatic expression of LPS-induced MIP-1α, MIP-1β, MCP-5, and RANTES mRNA occurs predominantly in Kupffer cells, while IP-10, MCP-1, and MIP-2 expression occurs within other liver cell types. These findings illustrate the complex nature of chemokine regulation and support the hypothesis that individual chemokines fulfill unique roles during the sepsis cascade.

Materials and Methods

Reagents

Protein-free (<0.008%), phenol-water-extracted *Escherichia coli* K235 LPS was prepared according to the method of McIntire et al. (26). Recombinant murine IL-10 and recombinant murine IFN-γ were provided by DNAx (Palo Alto, CA) and Genentech (San Francisco, CA), respectively. Liposomes that contain either dichloromethylene bisphosphonate (Cl2MBP; a gift of Boehringher Mannheim, Mannheim, Germany) or PBS were prepared as described previously (27).

**Mice**

C57BL/6J, C3H/HeJ, and C3H/OuJ mice (5–6 wk old) were purchased from The Jackson Laboratories (Bar Harbor, ME). IRF-1 and IRF-2 were obtained from The Jackson Laboratories (Bar Harbor, ME). IRF-1 and IRF-2/− mice were purchased from The Jackson Laboratories (Bar Harbor, ME). The mice used in these studies were the progeny of either homozygotic or heterozygotic matings as detailed elsewhere (29). The genotype of every mouse used in this study was confirmed as detailed previously (29). Stat1 and IRF-2 are Stat1 dependent, MIP-1, MIP-2, and KC is Stat1 dependent, and RANTES has been associated with LPS challenge. Serum was collected and stored at −70°C. JE/MCP-1, MIP-1α, and MIP-2 in serum and macrophage culture supernatants were measured by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. KC was measured in macrophage culture supernatants by ELISA using matched Ab pairs (rat anti-mouse KC IgG2A (1 μg/ml) and biotinylated goat anti-mouse KC IgG (0.5 μg/ml); R&D Systems) according to the manufacturer’s instructions. The lower limit of detection for JE/MCP-1, MIP-1α, MIP-2, and KC was 15.6, 4.7, 7.8, and 15.6 pg/ml, respectively.

Analysis of mRNA by RT-PCR

For peritoneal macrophage cultures, supernatants were removed after treatment and cells were lysed with RNA Stat-60 (Tel-Test, Friendswood, TX). For in vivo studies, organ tissue was harvested at indicated time points following treatment, frozen at −70°C, and later homogenized in RNA Stat-60. Total RNA was isolated as specified by the manufacturer and the concentration of RNA was determined by spectrophotometric analysis. The relative quantities of mRNA for each gene of interest was assessed by RT-PCR, followed by Southern blot analysis and detection by chemiluminescence, as detailed previously (34). The sequences for the PCR primers and probes used have been detailed elsewhere (25, 34, 35). The optimal cycle number for each gene in which detectable PCR-amplified product was achieved under nonsaturating conditions was determined empirically. Normalization for the relative quantity of mRNA was accomplished by comparison to either GAPDH or hypoxanthine-guanine phosphoribosyl transferase (HPRT), as previously described (34, 36). Increases in mRNA were expressed as mean-fold induction relative to untreated controls, which were arbitrarily assigned a value of 1. This precludes comparison of basal gene expression between organs, as well as among the different genes analyzed.

**Statistics**

Comparisons between two groups were analyzed using paired Student’s *t* test or ANOVA. The accepted level of significance was *p* < 0.05.

**Results**

Kinetic analysis of CXC and CC chemokine mRNA expression induced by LPS

To establish the time course of induction of chemokine genes in response to LPS, peritoneal macrophages from LPS-responsive C3H/OuJ mice were incubated with 200 ng/ml LPS from 0.5 to 48 h, and steady-state mRNA expression was measured by RT-PCR. Fig. 1 shows that mRNA expression of all chemokines, except RANTES, was induced 2- to 20-fold as early as 0.5 h after LPS treatment. Induction of RANTES mRNA above basal levels was not observed until 2 h post-LPS. KC, MIP-2, and MIP-1β mRNA reached peak levels (∼15- to 60-fold) as early as 1–2 h after LPS treatment, while IP-10, MIP-1α, RANTES, JE/MCP-1, and MCP-5 mRNA required 6 h before peak levels (∼10– to 90-fold) were achieved. In general, CXC and CC chemokine mRNA expression and function in vivo. Despite apparent functional redundancies and intrafamily structural homologies, these studies clearly demonstrate that individual chemokine genes are differentially regulated.
expression remained sustained for 48 h, with only a gradual decline in steady-state mRNA from peak levels. Steady-state mRNA expression for all of the CXC and CC chemokines was induced over basal levels by as little as 0.1 ng/ml LPS, and maximal mRNA expression was achieved with 1–10 ng/ml LPS (data not shown).

**IL-10-mediated suppression of LPS-induced chemokine mRNA expression**

Several studies have demonstrated the ability of IL-10 to downregulate LPS-inducible mRNA expression of proinflammatory cytokines (IFN-γ, IL-1, IL-6, IL-12, and TNF-α) (37–40) and chemokines (IP-10, KC, MIP-1α, and MIP-1β) (41–44) in macrophages. To determine whether the expression of different chemokine genes might be variably sensitive to IL-10, C3H/OuJ macrophages were incubated for 6 h with medium alone or LPS (1 ng/ml) in the absence or presence of IFN-γ (5 U/ml). Of the eight chemokine genes evaluated, treatment with IFN-γ alone was capable of inducing only MCP-5 (~20- to 25-fold) and IP-10 (~14-fold) mRNA expression (Fig. 3). IFN-γ inhibited LPS-induced MIP-1β and KC mRNA (~50–70%) and both mRNA and protein for MIP-1α, JE/MCP-1, and MIP-2 (Fig. 3 and Table II). In contrast, LPS-induced mRNA levels of RANTES, MCP-5, and IP-10 were unaffected by simultaneous treatment with IFN-γ.

**Induction of IP-10 and MCP-5 mRNA by LPS or LPS and IFN-γ is Stat1 dependent**

IP-10 and MCP-5 were the only two chemokine genes that were inducible by either IFN-γ or LPS alone. Because IFN-γ-mediated responses use activated Stat1 as a major transactivating factor, we sought to evaluate the role of Stat1 in LPS-induced chemokine gene expression. Thus, macrophages derived from wild-type and Stat1 knockout mice were compared for their ability to respond to LPS or LPS and IFN-γ. Of the eight chemokine genes examined, only LPS-induced IP-10 and MCP-5 mRNA expression was markedly abrogated in macrophages from Stat1−/− mice, while the other genes, like RANTES, were unchanged (Fig. 4). These data suggest a key role for Stat1 in the induction of IP-10 and MCP-5.

**Selective inhibition of LPS-induced chemokine mRNA expression by IFN-γ**

Like LPS, IFN-γ serves as a potent activating factor of macrophages. Although IFN-γ has been shown to interact synergistically with LPS to induce the mRNA for inflammatory mediators like TNF-α and inducible NO synthase (34, 45, 46), it also has been shown to suppress LPS-induced mRNA of other inflammatory mediators like MIP-1α, MIP-1β, JE/MCP-1, and KC (43). To investigate further the effect of IFN-γ on the panel of chemokines in this study, C3H/OuJ macrophages were incubated for 6 h with medium alone or LPS (1 ng/ml) in the absence or presence of IFN-γ (5 U/ml). Of the eight chemokine genes evaluated, treatment with IFN-γ alone was capable of inducing only MCP-5 (~20-to-25-fold) and IP-10 (~14-fold) mRNA expression (Fig. 3). IFN-γ inhibited LPS-induced MIP-1β and KC mRNA (~50–70%) and both mRNA and protein for MIP-1α, JE/MCP-1, and MIP-2 (Fig. 3 and Table II). In contrast, LPS-induced mRNA levels of RANTES, MCP-5, and IP-10 were unaffected by simultaneous treatment with IFN-γ.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>JE/MCP-1 (pg/ml)</th>
<th>MIP-2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>&lt;31</td>
<td>&lt;16</td>
</tr>
<tr>
<td>LPS (1 ng/ml)</td>
<td>6,387 ± 282</td>
<td>50,088 ± 7,251</td>
</tr>
<tr>
<td>LPS (1 ng/ml) + IL-10</td>
<td>1,822 ± 404</td>
<td>10,745 ± 1,890</td>
</tr>
<tr>
<td>LPS (10 ng/ml)</td>
<td>25,912 ± 3,800</td>
<td>89,521 ± 12,416</td>
</tr>
<tr>
<td>LPS (10 ng/ml) + IL-10</td>
<td>6,029 ± 289</td>
<td>29,393 ± 4,117</td>
</tr>
</tbody>
</table>

*Macrophages were treated with medium or LPS (1 or 10 ng/ml) in the absence or presence of IL-10 (100 U/ml). Supernatants were harvested at 6 h and analyzed for chemokine levels by ELISA.

The concentration of JE/MCP-1 and MIP-2 in the supernatants of LPS + IL-10-treated macrophages were significantly lower (p < 0.05) than those treated with LPS alone (n = 3).
IFN-γ-mediated inhibition of LPS-induced KC mRNA is dependent on IRF-2

The availability of mice deficient in the IFN-responsive transcription factor genes, IRF-1 and IRF-2, provided an opportunity to assess the role of these factors in the suppressive activity of IFN-γ on selected chemokine genes. Peritoneal macrophages from IRF-1+/− or IRF-2+/− mice were incubated for 6 h with medium alone or LPS (1 ng/ml) in the absence or presence of IFN-γ (5 U/ml). Data are expressed as the mean-fold induction ± SEM from three experiments. An asterisk with the percent reduction from LPS-stimulated values is included only when significantly different (p < 0.05). When not visible, error bars are smaller than the symbol.

Table II. The effect of IFN-γ on the production of JE/MCP-1, MIP-1α, and MIP-2 in culture supernatants of LPS-stimulated macrophages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>JE (pg/ml)</th>
<th>MIP-1α (pg/ml)</th>
<th>MIP-2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt. 1</td>
<td>Expt. 2</td>
<td>Expt. 1</td>
</tr>
<tr>
<td>Medium</td>
<td>75</td>
<td>28</td>
<td>&lt;9.4</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>59</td>
<td>33</td>
<td>&lt;9.4</td>
</tr>
<tr>
<td>LPS</td>
<td>4,114</td>
<td>2,260</td>
<td>4,428</td>
</tr>
<tr>
<td>LPS + IFN-γ</td>
<td>2,000</td>
<td>1,448</td>
<td>2,938</td>
</tr>
</tbody>
</table>

* Macrophages were treated with medium or LPS (1 ng/ml) in the absence or presence of IFN-γ (5 U/ml). Supematants were harvested at 6 h and analyzed for chemokine levels by ELISA.

FIGURE 3. Selective inhibition of IFN-γ on LPS-induced CC (A) and CXC (B) chemokine mRNA expression. CH12/OuJ macrophages were cultured for 6 h with medium alone or LPS (1 ng/ml) in the absence or presence of IFN-γ (5 U/ml). Data are expressed as the mean-fold induction ± SEM from three experiments. An asterisk with the percent reduction from LPS-stimulated values is included only when significantly different (p < 0.05). When not visible, error bars are smaller than the symbol.

FIGURE 4. The effect of Stat1 on the induction of LPS- and LPS plus IFN-γ-induced mRNA expression. Peritoneal macrophages from Stat1+/+ and Stat1−/− mice were cultured for 2 and 6 h with medium alone or LPS (1 ng/ml) in the absence or presence of IFN-γ (5 U/ml). Representative Southern blots of two independent experiments are shown.

LPS was neither IRF-1 nor IRF-2 dependent (data not shown). However, the ability of IFN-γ to suppress KC gene expression was entirely lost in macrophages from IRF-2−/− mice, while suppression of the other IFN-γ-sensitive chemokine genes (e.g., JE/MCP-1, MIP-2, MIP-1α, and MIP-1β) was unaffected by deletion of either gene (Fig. 5). Specifically, IFN-γ inhibited the LPS-induced KC mRNA expression (~72–84%) in IRF-2+/+ macrophages, while KC mRNA levels in IRF-2−/− macrophages were reduced only slightly. This derepression of LPS-induced KC mRNA by IFN-γ observed in IRF-2−/− macrophages was accompanied by elevated levels of KC protein in culture supernatants (Table III). In contrast, the IFN-γ-mediated inhibition of LPS-induced JE/MCP-1, MIP-1α, and MIP-1β mRNA was not reversed in IRF-1−/− or IRF-2−/− macrophages (Fig. 5 and data not shown). These data support a role for IRF-2 in IFN-γ-mediated inhibition of LPS-induced KC mRNA.

Differential expression of LPS-induced chemokine mRNA in vivo: role of Kupffer cells in vivo

To determine whether the pattern of LPS-inducible chemokines observed in vitro paralleled their induction in major shock organs, we measured the kinetics of hepatic chemokine mRNA expression in response to LPS (Fig. 6; open bars). All chemokine genes except RANTES were rapidly induced in the liver within 1 h of LPS challenge, and most reached peak levels (~50- to 100-fold) within 3 h of LPS administration. LPS-induced RANTES mRNA was delayed (3 h) and increased only ~20-fold. The kinetics of LPS-induced chemokine mRNA expression in the lung generally paralleled that observed in the liver, although reduced levels of pulmonary chemokine mRNA expression were observed for MIP-1β.
and MCP-5 (data not shown). Fig. 7 illustrates that MIP-2, JE/MCP-1, and MIP-1α were rapidly and substantially elevated in the serum within 1 h of LPS administration. Not only was the magnitude of JE/MCP-1 production the most profound, but it remained substantially elevated over baseline 48 h after LPS.

Although the data in Fig. 6 clearly shows that systemic administration of LPS to mice induces chemokine gene expression in the liver, the precise cell types that participate have not been clearly defined. To determine whether the Kupffer cell, the resident hepatic macrophage, is a predominant source of chemokines under these circumstances, we examined chemokine mRNA expression in livers from mice selectively depleted of Kupffer cells following i.v. administration of Cl₂MBP-liposomes (27, 32, 33, 47). Fig. 6 (solid bars) illustrates that induction of MIP-1α, MIP-1β, RANTES, and MCP-5 mRNA by LPS was reduced by 80–97% in the liver of Kupffer cell-depleted mice when compared with saline-pretreated, LPS-challenged control mice, indicating that macrophages and/or their secreted products are largely responsible for the expression of these genes in response to LPS. In contrast, the LPS-induction of MIP-2, IP-10, and JE/MCP-1 in the livers of macrophage-depleted mice were not reduced, but rather exhibited modest elevation 1–3 h after LPS challenge compared with control mice. However, by 6 h after LPS the level of MIP-2 mRNA from macrophage-depleted mice was not reduced, but rather exhibited modest elevation 1–3 h after LPS challenge compared with control mice. However, by 6 h after LPS when a slight decrease in mRNA from macrophage-depleted mice was detected. Because i.v. administration of Cl₂MBP-liposomes does not deplete pulmonary macrophages (27), chemokine mRNA expression in the lung was not examined.

Discussion

A hallmark of the inflammatory response to systemic LPS administration is the highly coordinated recruitment of leukocytes into host tissues. This process is mediated, in part, by the secretion of chemokines at sites of incipient inflammation. Based upon studies with cultured cells, virtually every cell type has the potential to generate large amounts of many chemokines. However, the pattern of chemokine expression in vivo appears to be much more selective. Furthermore, though many chemokines are expressed in inflamed tissues, individual chemokines have been demonstrated to play critical roles in endotoxemia and/or sepsis (20–24). However, to date most studies have focused on the role of individual chemokines in this process. In the present study, we have taken a more comprehensive and systematic approach to identify cellular and molecular mechanisms that may provide for such selective chemokine expression and function.

Because the interaction of LPS with macrophages is pivotal in eliciting the proinflammatory cascade associated with sepsis (48, 49), we initially focused on a characterization of the regulation of LPS-induced chemokine gene expression in macrophage cultures. We sought to identify stimuli (both pro- and antiinflammatory) in vitro that might account for differential expression in vivo. Furthermore, we wished to determine potential mechanisms operating in vivo that might account for distinct patterns of expression and/or function. Kinetic analyses of macrophage mRNA indicates that LPS rapidly induced (0.5 h) chemokine mRNA expression for all genes except RANTES (2 h), and, in contrast to the transient expression exhibited by most LPS-inducible early genes (36, 40, 50), chemokine steady-state mRNA levels remained elevated for 48 h. KC and MIP-2 both exhibited early (1–2 h) peak levels of mRNA expression, consistent with their role in the recruitment of neutrophils, the first leukocyte population to arrive at a site of inflammation. Likewise, chemokines responsible for recruiting subsequent leukocyte infiltrates, i.e., monocytes and T lymphocytes (i.e., IP-10, JE/MCP-1, MCP-5, MIP-1α, and RANTES), all exhibited delayed (6 h) peaks of mRNA expression. Thus, temporal regulation of macrophage chemokine expression may, in part, contribute

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>KC (pg/ml)</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>&lt;32</td>
<td>&lt;32</td>
<td>&lt;32</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>&lt;32</td>
<td>&lt;32</td>
<td>&lt;32</td>
</tr>
<tr>
<td>LPS</td>
<td>3596</td>
<td>3089</td>
<td>5538</td>
</tr>
<tr>
<td>LPS + IFN-γb</td>
<td>659</td>
<td>1873</td>
<td>948</td>
</tr>
<tr>
<td>(82%)</td>
<td>(39%)</td>
<td>(83%)</td>
<td>(43%)</td>
</tr>
</tbody>
</table>

a Macrophages were treated with medium or LPS (1 ng/ml) in the absence or presence of IFN-γ (5 U/ml). Supernatants were harvested at 6 h and analyzed for KC levels by ELISA.

b Percent reduction from LPS-stimulated value is included in parenthesis.
underscored by the increased lethality following neutralization of IL-10 in mice (23) and by the ability of exogenous IL-10 administration to prevent lethal shock in mice (53, 54). The antiinflammatory action of IL-10 has been attributed to its ability to inhibit transcription, to promote degradation of mRNA, and/or to reduce translation of numerous proinflammatory cytokines and chemokines (37, 41, 42, 55, 56). However, it has been recently demonstrated that IL-10 may suppress gene expression in stimulus-selective fashion (44) and we wished to determine whether differential sensitivity to IL-10 might be an important mechanism for generating differential expression of various chemokines. The LPS-induced chemokines previously reported to be sensitive to IL-10 inhibition include IP-10, KC, MIP-1α, and MIP-1β (41, 44). Our present findings confirm these observations and further demonstrate that IL-10 also inhibits LPS-induced MIP-2, JE/MCP-1, MCP-5, and RANTES mRNA expression, although RANTES and MIP-1β were considerably less sensitive to inhibition by IL-10, particularly at higher doses of LPS. Collectively, these findings support a novel role for IL-10 as a key antiinflammatory agent in vivo: As a result of IL-10’s inhibition of the panel of eight LPS-induced chemokine genes examined, leukocyte infiltration (and subsequent release of proinflammatory mediators) into tissues would likely be attenuated.

In contrast to the broad antiinflammatory actions of IL-10, IFN-γ is generally considered to be a potent macrophage activator that interacts synergistically with LPS to induce inflammatory mediators like TNF-α and iNOS (34, 45, 46), in addition to enhancing LPS-induced lethality (57–59). LPS-induced MCP-5 and IP-10, genes which are inducible by IFN-γ alone, and RANTES were unaffected by cotreatment with LPS and IFN-γ. However, IFN-γ has been shown to antagonize induction of JE/MCP-1, KC, MIP-1α, and MIP-1β gene expression in LPS-stimulated macrophages (43, 60), and the present study extends this to include MIP-2. Thus, the differential action of IFN-γ on individual chemokine genes provides an example of selective stimulus sensitivity that may be important in mediating the highly restricted patterns of chemokine gene expression noted in vivo.

The availability of mice in which transcription factors that mediate responses to IFNs have been deleted by gene targeting provides the opportunity to explore further the mechanisms involved in the regulation of LPS-induced chemokine expression. Activated Stat1α homodimers are the primary transcriptional activation complex formed by IFN-γ-stimulated cells and are necessary for the expression of IP-10 in response to IFN-γ (61). Because IP-10 and MCP-5 are inducible with either IFN-γ or LPS, we wished to determine whether Stat1 were also necessary for chemokine expression in response to LPS. Analysis of the LPS inducibility of all of the chemokine genes examined revealed that only IP-10 and MCP-5 were strongly dependent on Stat1 for their induction by LPS.

Both IFN-γ and LPS also activate expression of two other well-characterized IFN-responsive DNA binding proteins, IRF-1 and IRF-2 (36, 62). IRF-1 serves predominantly as a transcriptional activator (63), whereas IRF-2 functions generally as a transcriptional repressor (64–66). Although neither IRF-1 nor IRF-2 were required for induction of the chemokine genes examined, our studies also revealed a novel role for IRF-2 in the IFN-γ-mediated inhibition of LPS-induced KC, but not of other IFN-γ-inhibitable genes. Promoter analysis of the murine KC gene has revealed two NF-kB motifs that allow for its induction by LPS; however, no functional IFN-responsive regulatory sequences have been defined (67). Nevertheless, it is possible that the requirement for IRF-2 in negative transcriptional regulation of the KC gene by IFN-γ is not mediated through interaction with an IFN-responsive sequence.

FIGURE 6. Kinetics of CXC (A) and CC (B) chemokine mRNA expression in the livers of control (open bars) and Kupffer cell-depleted (solid bars) C57BL/6J mice after LPS (25 μg) challenge. Data are expressed as the mean ± SEM from nine individual mice. The means are expressed relative to the response of untreated mice, which are assigned a value of 1. When not visible, error bars are smaller than the symbol.

FIGURE 7. Production of MIP-2, MIP-1α, and JE/MCP-1 in the serum of C57BL/6J mice after LPS (25 μg) challenge. Data are expressed as the mean ± SEM from four individual mice. When not visible, error bars are smaller than the symbol.

FIGURE 8. Production of MIP-2, MIP-1α, and JE/MCP-1 in the serum of C57BL/6J mice after LPS (25 μg) challenge. Data are expressed as the mean ± SEM from four individual mice. When not visible, error bars are smaller than the symbol.
motif. In support of this possibility is the recent finding that both IRF-1 and IRF-2 can interact with NF-kB to control the transcriptional regulation of the MHC class I gene (68), as well as others. Even though KC was the only LPS-inducible chemokine gene to exhibit IRF-2-dependent, IFN-γ-mediated inhibition, it is not the only gene that we have found to exhibit this pattern of regulation: IFN-γ-mediated suppression of LPS-induced IL-12 p35 mRNA expression is also dererepressed in macrophages derived from IRF-2−/− mice (69). The molecular basis for the role of IRF-2 in this process will require further analysis. Nonetheless, the finding that no other IFN-γ-inhibited, LPS-induced chemokine gene was regulated by either IRF-1 and IRF-2 further highlights the exquisite differential regulation of these genes.

Our in vivo results demonstrate that the entire panel of CXC and CC chemokines (with the exception of RANTES) were also rapidly induced in the lung and liver after LPS administration. The pattern of chemokine mRNA induction that we observed in the tissue following LPS challenge was parallel by rapid and sustained production of chemokines (i.e., JE/MCP-1, MIP-1α, and MIP-2) in the serum (Figs. 6 and 7). Although elevated serum chemokine levels may be required to sustain leukocyte mobilization from the circulation, as well as from the bone marrow, over the duration of an inflammatory response, the profoundly elevated and sustained levels of JE/MCP-1 in the serum may reflect its recently discovered role as an antiinflammatory mediator (20). Although the influx of neutrophils typically precedes that of monocytes and lymphocytes into tissues (24), in our in vivo data do not support the hypothesis that this process is regulated by production of neutrophil chemotactants before production of monocyte and lymphocyte chemotactants, as suggested by our in vitro data that demonstrated a temporal staggering of neutrophil vs monocyte chemotacticant mRNA expression. Thus, the rapid influx of neutrophils to the site of inflammation is likely to be controlled by additional factors such as the relative abundance of neutrophils in the bloodstream or the differential modulation of other mediators of cellular trafficking, such as adhesion molecules and chemokine receptors.

The selective depletion of Kupffer cells by Cl2MBP-liposome treatment (27, 32, 33, 47) allowed us to assess the relative contribution of macrophages in the induction of chemokine mRNA in the liver following LPS challenge. To our knowledge, Fig. 6 provides the first direct evidence that macrophages and/or their secreted products contribute significantly to the induction of MIP-1α, MIP-1β, RANTES, and MCP-5 mRNA in the liver following LPS challenge. The observation that macrophages are the major source of certain chemokines is consistent with a previous report that identified macrophages as the primary source of MIP-1α in the lungs of LPS-challenged mice (24). Conversely, cell types apart from Kupffer cells produce IP-10, JE/MCP-1, KC, and MIP-2 mRNA in the livers of LPS-challenged, macrophage-depleted mice. The observed elevated expression of IP-10, JE/MCP-1, and MIP-2 mRNA in macrophage-depleted mice may reflect the absence of a negative regulator like IL-10, which is ablated in the liver by macrophage depletion (33).

In summary, we have identified molecular and cellular mechanisms that may contribute to differential patterns of chemokine expression in vivo. The specific pattern of expression of each individual chemokine is likely to reflect its unique function in the regulation and progression of an inflammatory response. The complex orchestration of chemokines in response to LPS highlights the necessity for understanding the timing and localization of chemokine expression and production to identify potential targets for therapeutic intervention.

Acknowledgments

We thank Annemarie Sanders for preparation of liposomes and Ronney McNally, Diana Miller, and Gregory Detore for excellent technical assistance.

References