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Expression and Contribution of Endogenous IL-13 in an Experimental Model of Sepsis

Akihiro Matsukawa,* Cory M. Hogaboam,* Nickolas W. Lukacs,* Pamela M. Lincoln,* Holly L. Evanoff,* Robert M. Strieter,† and Steven L. Kunkel2*  

IL-13 has been shown to exert potent anti-inflammatory properties. In this study, we elucidated the functional role of endogenous IL-13 in a murine model of septic peritonitis induced by cecal ligation and puncture (CLP). Initial studies demonstrated that the level of IL-13 increased in tissues including liver, lung, and kidney, whereas no considerable increase was found in either peritoneal fluid or serum after CLP. Immunohistochemically, IL-13-positive cells were Kupffer cells in liver, alveolar macrophages in lung, and epithelial cells of urinary tubules in kidney. IL-13 blockade with anti-IL-13 Abs significantly decreased the survival rate of mice after CLP from 53% to 14% on day 7 compared with control. To determine the potential mechanisms whereby IL-13 exerted a protective role in this model, the effects of anti-IL-13 Abs on both local and systemic inflammation were investigated. Administration of anti-IL-13 Abs did not alter the leukocyte infiltration and bacterial load in the peritoneum after CLP but dramatically increased the neutrophil influx in tissues after CLP, an effect that was accompanied by significant increases in the serum levels of aspartate transaminase, alanine transaminase, blood urea nitrogen, and creatinine. Tissue injury caused by IL-13 blockade was associated with increases in mRNA and the protein levels of CXC chemokines macrophage inflammatory protein-2 and KC as well as the CC chemokine macrophage inflammatory protein-1α and the proinflammatory cytokine TNF-α. Collectively, these results suggest that endogenous IL-13 protected mice from CLP-induced lethality by modulating inflammatory responses via suppression of overzealous production of inflammatory cytokines/chemokines in tissues. The Journal of Immunology, 2000, 164: 2738–2744.

Sepsis and septic syndrome represent an intense systemic response with multiple physiological and immunological abnormalities that is commonly caused by bacterial infection (1, 2). The manifestation of sepsis initially may be caused by intense local inflammation, the frequent sites of which are lung, urinary tract, and abdomen (1, 2). Once the host fails to restrict the invading pathogens to a localized area of tissue, an overwhelming systemic response may occur via the overzealous production of inflammatory mediators including cytokines and chemokines, leading to systemic inflammatory response syndrome (3) or multiple organ failure (3, 4). Therefore, the host’s response toward the pathogens must be under strict regulation because the consequence of uncontrolled inflammation can be more fatal than the original inciting pathogens. Recent evidence suggests that the deleterious effects of inflammatory cytokines are counterbalanced by endogenous anti-inflammatory cytokines such as IL-10 (3–5).

IL-13, like IL-10, has been shown to exert potent anti-inflammatory properties (6, 7). In vitro, IL-13 modulates monocyte-derived TNF-α, IL-1, and IL-8 while stimulating the production of IL-1 receptor antagonist and increasing the expression and release of the IL-1 type II decoy receptor (6–9). Recent experiments in a murine model of endotoxemia in which exogenous IL-13 treatment protected mice from lethal endotoxemia by reducing systemic production of TNF-α confirmed the anti-inflammatory properties of IL-13 in vivo (10–12). However, no increase in IL-13 levels has been detected in plasma of patients with sepsis or endotoxemic volunteers (13). Therefore, unlike with known immune-regulating cytokines like IL-10, it is not known whether endogenous IL-13 actually functions as a host defense mechanism during the evolution of sepsis. To our knowledge, there is no report describing the in vivo functional role of endogenous IL-13 in human sepsis or in experimental models of endotoxemia and sepsis.

The aim of the present study was to elucidate the functional role of endogenous IL-13 in experimental sepsis. For this purpose, we employed a murine model of septic peritonitis induced by cecal ligation and puncture (CLP),3 which possesses a number of the hallmarks of clinical sepsis with peritonitis associated with postsurgical or accidental trauma (14). Using this model, we have found that IL-13 is expressed rapidly post-CLP in tissues including liver, lung, and kidney. Interestingly, no increased level of this mediator was found in either the peritoneal fluid or serum. The contribution of IL-13 to the evolving septic response was assessed by passive immunization with anti-IL-13 Abs. These studies have demonstrated that IL-13 is an important cytokine that is involved in regulating organ-specific inflammation by controlling the production of tissue levels of specific cytokines and chemokines.

Materials and Methods

Cecal ligation and puncture

Female CD-1 mice (6–8 wk of age) were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed for at least 7 days before manipulations. The mice were subjected to CLP surgery, as described previously (5, 14). In brief, the mice were anesthetized with i.p. injection of ketamine HCl (Vetamine, Mallinckrodt Veterinary, Mundelein, IL) before inhaled methoxyflurane (Metofane; Mallinckrodt Veterinary). Under sterile

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3 Abbreviations used in this paper: CLP, cecal ligation and puncture; MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; MPO, myeloperoxidase; AST, aspartate transaminase; ALT, alanine transaminase; BUN, blood urea nitrogen.
conditions, the cecum was exposed through a 1- to 2-cm incision of the lower left abdomen, it was ligated with a 3-0 silk suture below the ileocecal valve, and then it was punctured through and through once with a 21-gauge needle. The cecum was replaced in the peritoneum and the abdomen was closed with surgical staples. The mice were injected with 1 ml of saline s.c. for fluid resuscitation and were placed on a heating pad until they recovered from anesthesia.

Neutralization of IL-13

Passive neutralization of IL-13 was conducted by i.p. injection of 0.5 ml of anti-murine IL-13 antiserum twice, 2 h before CLP and 2 days after CLP. The volume of antiserum was considered to be sufficient to neutralize systemic endogenous IL-13 in that the biological half-life of the Ab was ~36 h (C. M. Hogaboam, unpublished observation). Anti-murine IL-13 polyclonal Abs were raised by immunizing New Zealand White rabbits with recombinant murine IL-13 (R&D Systems, Minneapolis, MN). Polyclonal Abs were titrated by direct ELISA and these Abs recognized murine IL-13 at a dilution of $1 \times 10^{-5}$. The Abs completely blocked the ability of IL-13 to inhibit the production of TNF-$\alpha$, macrophage inflammatory protein (MIP)-2, and MIP-1$\alpha$ by LPS-stimulated macrophages in vitro and did not cross-react with other murine recombinant cytokines and chemokines. As a control, preimmune rabbit serum was used. The endotoxin content in both anti-IL-13 antiserum and control serum was below detection level (<0.05 EU/ml PYROGENT; BioWhittaker, Walkersville, MD).

Experimental protocol

In the first set of experiments, mice were monitored for 7 days after CLP to determine the mortality rate induced with CLP. In the next set of experiments, the CLP mice were anesthetized at specific time points, euthanized, and bled. The peritoneal cavities were washed with 2 ml of sterile saline, and the lavage fluids were collected. After taking a 10-$\mu$l aliquot of lavage fluids for assessment of bacteria colony-forming units, the fluids were centrifuged at 6000 x g for 1 min at 4°C, and cell-free peritoneal fluids were stored at $-20^\circ$C. Cell pellets were resuspended in saline, and the cell numbers were counted in a hemocytometer; in addition, differential cell analysis was made after Diff-Quik staining of the smear slides (Dade, Düdingen, Switzerland). The liver, lung, and kidney were excised, weighed, frozen in liquid nitrogen, and stored at $-20^\circ$C for subsequent analyses.

Determination of CFU

Ten microliters of peritoneal lavage fluids and peripheral blood from each mouse were placed on ice and were serially diluted with sterile saline. Ten microliters of each dilution were plated on thymic-sharing Ag blood agar plates (Difco, Detroit, MI) using standardized techniques.

Clinical chemistry

Serum levels of aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN), and creatinine were measured by Clinical Pathology at the University of Michigan Medical School (Ann Arbor, MI) using standardized techniques.

Measurement of cytokines and myeloperoxidase (MPO)

Murine IL-13 was quantitated using a standard method of sandwich ELISA. In brief, microtiter plates (Nunc Immuno-Plate I 96-F; Nunc, Roskilde, Denmark) were coated with 50 $\mu$l of affinity-purified anti-murine IL-13 IgG (1 $\mu$g/ml) in coating buffer (0.6 M NaCl, 0.26 M H$_2$BO$_4$, and 0.08 M NaOH (pH 9.6)). Detection and processing were made by using biotinylated rabbit anti-murine IL-13 IgG (3.5 $\mu$g/ml), streptavidin-peroxidase conjugate (Bio-Rad, Richmond, CA), and chromogen substrate (Bio-Rad). The protein concentrations of murine TNF-$\alpha$, IL-10, MIP-1$\alpha$, MIP-2, KC, and monocyte chemotactic protein (MCP)-1 were measured by specific ELISAs, as previously described in detail (5, 15). The ELISAs employed in this study did not cross-react with other murine cytokines and consistently detected murine cytokine concentrations above 25 pg/ml.

MPO in tissue extracts was measured by using an ELISA kit (Calbiochem-Novabiochem, San Diego, CA) according to the manufacturer’s instruction. The lower detection limit was 1.6 ng/ml.

Preparation of tissue extracts

Excised tissues (0.1 g) were placed in 1 ml of homogenization buffer (500 mM NaCl and 50 mM HEPES (pH 7.4) containing 0.1% Triton X-100, 0.5 $\mu$g/ml leupeptin, 1 mM PMSF, and 0.02% NaN$_3$) and homogenized with a Tissue Tearor (model 985-370; Biospec Products, Racine, WI). The homogenates subsequently received freeze/thaw extraction once for ELISA and three times for MPO assay. The homogenates were centrifuged at 6000 x g for 20 min at 4°C, and the cleared supernatants were used for measurement of cytokines or MPO.

Immunohistochemistry

Liver, lung, and kidney were excised at 24 h after CLP surgery, fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin, and 4-$\mu$m thin sections were prepared. Alveolar macrophages, harvested at 24 h after CLP, were sedimented at $1 \times g$ onto BSA-coated glass slides, air-dried, and fixed in 100% ethanol for 10 min. Immunostaining was conducted using Dako EnVision System (Dako, Carpinteria, CA) according to the manufacturer’s instructions. In brief, endogenous peroxidase activity was blocked with 0.3% H$_2$O$_2$ in methanol, and the sections and smear slides were incubated for 30 min with 1000 $\times$ diluted anti-murine IL-13 anti- serum at room temperature. The specimens were rinsed and then incubated for 30 min with peroxidase-labeled polymer (Dako) at room temperature. As a chromogen, diaminobenzidine (Dako) was used. Counterstaining was performed with hematoxylin. Diluted preimmune serum (1000 $\times$) was used as a control.

RT-PCR

The tissues were homogenized in Trizol Reagent (Life Technologies, Grand Island, NY), and total RNA was isolated according to the manufacturer’s instructions. First-strand cDNA was constructed from 2 $\mu$g of total RNA with oligo(dT)$_{12-18}$ as primers, and the first-strand cDNAs were then amended by each PCR in the presence of Taq polymerase (Life Technologies) and specific primers. The primers were designed to amplify murine cytokines/chemokines referred to the cDNA sequence from the National Center for Biotechnology Information database. The primers are as follows. MIP-2: sense, 5'-GCTGCCACCAAACCCACACCAG-3'; anti-sense, 5'-AGCGAGGCACATCAGGTACG-3'; KC: sense, 5'-TGAGCT GGCCTGTACAGCTC-3'; anti-sense, AGAACGACGTCACCAAG3'; MIP-1$\alpha$: sense, 5'-GCCCCCTTCTTCTCTCTCCTG-3'; anti-sense, 5'- GGCAATCAATTCAGGTAGCTG-3'. TNF-$\alpha$: sense, 5'-CTTCCAGAA
CTCAGGGCGGT-3'; antisense, 5'-GAGGAGGTTGACTTTCTCCT-3'. GAPDH: sense, 5'-GGTGAAGGTCGGTGTCAACGGATTT-3'; antisense, 5'-GATGCCAAAGTTGTCATGGATGACC-3'. The PCR reaction was conducted at 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Twenty microliters of each PCR product was subjected to electrophoresis on a 2% agarose gel in the presence of ethidium bromide and photographed, then the images were digitized, and the band densities were measured with NIH Image. Results are expressed as a ratio of each PCR product/GAPDH band density, and this represents semiquantitative analysis.

Statistics

Statistical significance was determined by ANOVA with p values <0.05. In case of survival curve and CFU count, the data were analyzed by the log-rank test and Mann-Whitney test, respectively. A p value <0.05 was regarded as statistically significant. All data were expressed as the mean ± SD.

Results

Detection of IL-13 in the development of CLP-induced sepsis

In initial investigations, we first measured the local and systemic levels of IL-13 during the evolution of CLP-induced sepsis. The expression of IL-13 in the liver, lung, and kidney was significantly increased after CLP (Fig. 1); however, there was not a significant increase in the levels of IL-13 in the peritoneal fluid or serum after CLP (data not shown). IL-13 levels in liver and kidney reached a peak at 4 and 8 h post-CLP, respectively, and remained elevated at 48 h, whereas IL-13 levels in the lung gradually increased with time (Fig. 1). The peak levels of IL-13 in liver, lung, and kidney were 3.2-, 5.0-, and 6.0-fold higher, respectively, than those in normal tissue. To identify the cells expressing IL-13 in tissues, immunohistochemical staining was performed. As shown in Fig. 2, Kupffer cells in the liver and epithelial cells in the urinary tubules stained positive in tissue sections obtained at 24 h post-CLP (Fig. 2, A and E). IL-13-positive cells in lung tissue section could not be determined, but alveolar macrophages collected by lavage at 24 h after CLP were positively stained (Fig. 2C). Control Abs were not reactive, indicating the specificity of the staining (Fig. 2, B, D, and F). The above data indicate that IL-13 levels were augmented only in tissues during the evolution of CLP-induced peritonitis.

Neutralization of IL-13 increases CLP-induced lethality

To examine the involvement of IL-13 in CLP-induced peritonitis leading to lethality, either anti-IL-13 Abs or control Abs were i.p. injected 2 h before CLP surgery and 2 days after CLP, and the survival rates were monitored. As shown in Fig. 3, neutralization of IL-13 significantly decreased the survival of mice after CLP. The detrimental effects were most apparent at 48 h, when the mortality rate in control mice was 30% (9/30 mice), whereas a striking 64% mortality rate (18/28 mice) was observed in mice treated with anti-IL-13 Abs (Fig. 3). On day 7, 53% of control mice (16/30 mice) survived compared with 14% of anti-IL-13 Ab-treated mice (4/28 mice).

Because IL-4 shares a number of similar biological properties with IL-13, presumably due to both a common receptor component and signaling pathway (16, 17), we compared the contribution of IL-4 to IL-13 using IL-4-neutralizing Abs. Neutralization of IL-4 had no effect on the survival rate after CLP (Fig. 3), suggesting that either endogenous IL-4 does not function in the same manner as...
IL-13 or IL-4 is not produced in significant quantities in this model. In fact, no significant increases were found in the level of IL-4 in the peritoneum, serum, or tissues after CLP. The above results do clearly indicate a protective role of endogenous IL-13 but not IL-4 in CLP-induced lethality.

IL-13 blockade does not affect the local inflammation induced by CLP

To identify the mechanisms whereby IL-13 exerted a protective role in CLP, experiments were conducted to assess the local effects of anti-IL-13 Abs. Blockade of IL-13 resulted in no change in the numbers of either infiltrating neutrophils or macrophages in the peritoneum at any time point after CLP, compared with control (data not shown). Production of chemokines that attract leukocytes in the peritoneum, which include MIP-2, KC, MIP-1α, and MCP-1, increased after CLP, but these levels were unchanged by anti-IL-13 Abs, compared with control (data not shown). Because activated leukocytes phagocytose and eliminate bacteria (18), we determined the phagocytic index of the peritoneal leukocytes by assessing the bacteria load in the peritoneum after CLP. At 24 h after CLP, the recovery of bacteria from the peritoneum was found in 9/10 mice treated with control Abs, and the mean CFU was $1.2 \times 10^7/10$ μl of peritoneal fluids. At this same time point, similar levels of viable bacteria were recovered from the peritoneum of mice that received anti-IL-13 Abs (9/9 mice; mean CFU = $0.9 \times 10^7/10$ μl, not significant compared with control).

Thus, it appears that endogenous IL-13 does not play a role in regulating the phagocytic and bactericidal activity of the infiltrating leukocytes.

IL-13 blockade augments systemic tissue inflammation and tissue injury induced by CLP

Next, experiments were conducted to assess the systemic effects of anti-IL-13 Abs. To examine systemic inflammation, we measured the level of MPO in tissues as an indirect means to determine the recruitment of neutrophils. Neutralization of IL-13 resulted in a significant increase in MPO levels in the liver and kidney compared with control (Fig. 4). MPO levels in the lung were also increased by 30% after anti-IL-13 treatment, although the difference was not statistically significant (control vs anti-IL-13 Abs; $29.5 \pm 4.2$ ng/0.1 g lung ($n = 9$) vs $39.1 \pm 3.8$ ng/0.1 g lung ($n = 9$); $p = 0.106$; Fig. 4). Treatment with anti-IL-13 Abs also significantly increased serum levels of AST, ALT, BUN, and creatinine compared with control (Fig. 5). The data indicate that endogenous IL-13 modulates systemic tissue inflammation and tissue dysfunction evolved by CLP.

Neutralization of IL-13 alters the production of MIP-2, KC, MIP-1α, and TNF-α in tissues

To elucidate the mechanisms whereby anti-IL-13 treatment increased the tissue inflammation during the evolution of CLP, the levels of chemokines known to attract neutrophils, which include

**FIGURE 3.** IL-13 blockade reduces survival of mice after CLP. A total of 0.5 ml of anti-IL-13 antiserum (○; 28 mice), anti-IL-4 antiserum (■; 34 mice), or control serum ( []; 30 mice) was i.p. injected 2 h before CLP and 2 days after CLP. The survival rates were monitored for 7 days after CLP. There is a statistically significant difference ($p < 0.01$) between anti-IL-13 antiserum and control, whereas there is no significant difference between anti-IL-4 antiserum and control (log-rank test).

**FIGURE 4.** Effects of anti-IL-13 antiserum on the MPO level in tissues. A total of 0.5 ml of either anti-IL-13 antiserum (■; 10 mice) or control serum ( []; 9 mice) was i.p. injected 2 h before CLP. At 24 h after CLP, mice were euthanized, and the liver, lung, and kidney were resected. The tissues were extracted and the amounts of MPO in liver, lung, and kidney were measured by ELISA. The data represent the mean ± SD. ‡, $p < 0.05$; ††, $p < 0.0001$, compared with control. Dotted lines represent the mean data obtained from normal tissues (6 mice).

**FIGURE 5.** Effects of anti-IL-13 antiserum on the clinical chemistry after CLP. A total of 0.5 ml of either anti-IL-13 antiserum (■; 10 mice) or control serum ( []; 9 mice) was i.p. injected 2 h before CLP. At 24 h after CLP, mice were euthanized and bled, and the sera were harvested. The amounts of AST, ALT, BUN, and creatinine in the sera were measured. The data represent the mean ± SD. †, $p < 0.01$; ¶, $p < 0.001$, compared with control. Dotted lines represent the mean data obtained from normal serum (6 mice).
MIP-2, KC, and MIP-1α, were next examined after anti-IL-13 treatment. The levels of these chemokines increased after CLP and peaked at 8–24 h after CLP (data not shown). Neutralization of IL-13 resulted in significant increases in the level of CXC chemokines MIP-2 and KC in liver, lung, and kidney compared with controls. As shown in Fig. 6, anti-IL-13 treatment markedly increased the levels of MIP-2 and KC in tissues at 8 h after CLP. Twenty-four hours after CLP, the levels of MIP-2 in the lung and kidney and the level of KC in the kidney were still higher than those in control (Fig. 6A). The mRNA expressions for MIP-2 and KC in all tissues examined were up-regulated by anti-IL-13 Abs compared with control (Fig. 6, B and C). The level of CC chemokine MIP-1α in the lung remained elevated after 24 h for anti-IL-13-treated mice but decreased at 24 h in control mice compared with the level at 8 h (Fig. 7A). MIP-1α in the kidney was increased at 8 h after the treatment with anti-IL-13 Abs, and the significant increase was still found at 24 h after CLP (Fig. 7A). We also assessed the production of proinflammatory cytokine TNF-α, which is known to be involved in the pathogenesis of sepsis (3), in our model of sepsis. The levels of TNF-α in tissues after CLP increased with time for up to 24 h (data not shown). Neutralization of IL-13 significantly increased the level of TNF-α in the liver (24 h) and kidney (8 and 24 h) but not in the lung, compared with control (Fig. 7A). The mRNA expressions for MIP-1α in the lung (18 h after CLP) and kidney (3 and 18 h) and for TNF-α in the liver (18 h) and kidney (3 and 18 h) were up-regulated by anti-IL-13 Abs (Fig. 7, B and C). Thus, the in vivo neutralization of IL-13 increased the gene expression and protein production of neutrophil-activating chemokines and proinflammatory cytokine (TNF-α) in specific tissues.

Discussion

The systemic inflammatory response induced by CLP is known to be balanced by a set of regulatory cytokines (3–5); however, if this fine balance is lost, the inflammatory response can become pathological, self-destructive, and fatal to the host (19). The fact that treatment with exogenous IL-13 alters the cytokine balance in favor of anti-inflammatory effects both in vitro and in vivo led to the assumption that this cytokine might function as a potent endogenous immunomodulator in inflammatory diseases (6–12). Recent studies demonstrate that IL-13 can modulate the intensity of lung inflammation induced by the IgG immune complex (20). In our model of sepsis, neutralization of IL-13 was detrimental to the survival of mice after CLP, indicating that the contribution of endogenous IL-13 is to serve as a protective cytokine during the evolution of septic peritonitis.

Sepsis induced by CLP initiates in the peritoneum, where the local inflammatory response is essential for eliminating invading pathogens from the infectious foci. Previously, IL-13 has been shown to inhibit the production of inflammatory cytokines/chemokines in vitro and in vivo (6, 7, 10–12). Administration of IL-13 reduced the recruitment of leukocytes in a model of chronic, Ag-induced guinea pig lung inflammation (21). In contrast, the neutralization of IL-13 in IgG immune complex-induced lung inflammation enhanced leukocyte infiltration by altering inflammatory cytokine/chemokine production (20). Because many inflammatory cytokines (including chemokines) are produced in the peritoneum stained PCR products were photographed, and then the images were digitized and analyzed. Results are expressed as a ratio of each PCR product/ GAPDH band density. The data in this figure represent three independent experiments. PCR was performed in duplicate for each experiment.
after CLP, we speculated that endogenous IL-13 might directly modulate local inflammatory reactions after this insult. However, neither leukocyte infiltration nor bacterial load in the peritoneum after CLP was altered after the treatment with anti-IL-13 Abs. Likewise, the production of chemokines in the peritoneum that attracts leukocytes, which include MIP-2, KC, MIP-1α, and MCP-1, was unchanged by anti-IL-13 treatment. The findings are not surprising because no significant increase in IL-13 was found in the peritoneum after CLP throughout the observation periods. The data indicate that IL-13 may not be involved in the regulation of local inflammation in the CLP model with respect to chemokine production, leukocyte infiltration, and activation of the leukocytes.

Sepsis is known to frequently cause severe systemic inflammation called systemic inflammatory response syndrome, which leads to multiple organ failure, a condition that is often fatal to the host (3, 4). Interestingly, the level of IL-13 only increased in tissues after CLP without any increase in the level of IL-13 in the peritoneum fluids and serum, which correlates with a recent clinical report showing that IL-13 did not increase in the plasma of patients with sepsis and endotoxemic volunteers (13). Although the failure to find increased IL-13 in the serum and peritoneal fluids may be a consequence of the presence of a soluble receptor/receptor antagonist against IL-13, the clinical and experimental findings suggest that endogenous IL-13 exerts a protective role by modulating specific organ/tissue inflammation after CLP. This is likely the mechanism underlying the protective role of endogenous IL-13 in CLP animals in that IL-13 blockade not only augmented the intensity of the tissue inflammation but also impaired the physiological function of specific tissues. The modulation of tissue inflammation by endogenous IL-13 appeared to be mediated by down-regulating chemokine production in tissues, as shown by the fact that the mRNA and protein levels of MIP-2, KC, and MIP-1α in specific tissues were dramatically increased after anti-IL-13 treatment compared with control. These chemokines are known to be responsible for various types of tissue inflammation including sepsis (2, 3, 22, 23). Furthermore, neutralization of IL-13 increased both the mRNA and protein levels of TNF-α in tissues in our model of sepsis. The data corresponded well to the previous reports that showed that IL-13 inhibited TNF-α production in vitro and in vivo (6, 7, 10–12). TNF-α is known to induce the expression of many types of mediators including chemokines, and is a powerful pathogenic mediator of tissue injury (24, 25).

The protective role of endogenous IL-13 appeared to be independent of another anti-inflammatory cytokine IL-4 and IL-10. No appreciable increase in IL-4 was found during sepsis induced by CLP, and neutralization of IL-4 had no effect on the survival of mice after CLP. We and others demonstrated that CLP led to a rapid production of IL-10 during sepsis (5, 26) and that IL-10 blockade was detrimental to the survival of mice after CLP, which was associated with increased level of TNF-α (26, 27), indicating that endogenous IL-10 plays a protective role during CLP-induced sepsis. Because IL-13 has been shown to directly inhibit macrophage IL-10 production in vitro (28) and to decrease the level of IL-10 in lethal endotoxemia (10), we asked whether IL-13 blockade would affect the production of IL-10 in our model of sepsis. As a result, neutralization of IL-13 failed to alter the level of IL-10 in tissues after CLP (data not shown). The data suggest that IL-13 provided a protection mechanism that is similar to but independent of IL-10.

Although IL-13 was originally identified as a product of activated Th2 cells (29, 30), recent investigations have shown that IL-13 can be produced in vitro from many types of cells such as mast cells, basophils, NK cells, fibroblasts, dendritic cells, PBMCs, or alveolar macrophages (31–37). In CLP animals, IL-13...
was immunohistochemically detected in liver Kupffer cells, lung alveolar macrophages, and kidney epithelial cells in urinary tubules. There are other possible sources of IL-13 in the lung such as mast cells, NK cells, or T cells; however, we believe that alveolar macrophages are the main cells producing IL-13 in the lungs of animals undergoing CLP.

The data obtained in this study demonstrate the beneficial effects of endogenous IL-13 in the septic peritonitis. The levels of IL-13 were increased in the liver, lung, and kidney during the evolution of CLP, and neutralization of endogenous IL-13 resulted in an enhanced expression and production of inflammatory cytokines/chemokines, augmented tissue inflammation, and induced tissue dysfunction. Thus, it appears that endogenous IL-13 protected mice from CLP-induced lethality by modulating systemic inflammatory responses via suppression of the overzealous production of tissue-specific inflammatory cytokines and chemokines.

Acknowledgments

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