CpG oligodeoxynucleotides (ODNs) may prevent mortality from infection. We have identified a therapeutic benefit in treating sepsis with phosphorothioate ODN sequences containing the CpG motif. Sepsis was induced in rats by cecal ligation and puncture (CLP), and treatment with CpG ODNs reduced sepsis mortality from 80% to 15% during a 108-h period. Protection from mortality was dose dependent. Bacterial load in peritoneal fluid was reduced in CpG ODN–treated versus non-CpG ODN–treated rats after CLP. Lung injury, as determined by total myeloperoxidase activity, was also reduced in CpG ODN–treated versus non-CpG ODN–treated rats after CLP. Indirect evidence suggests that CpG-induced expression of interleukin (IL)–23 as levels of p40—but not p35—were significantly increased in both plasma and peritoneal lavage fluid in CpG ODN–treated versus non-CpG ODN–treated rats 24 h after CLP. Anti–IL-17 antibody inhibited the CpG-mediated prevention of mortality. These data suggest that IL-17 may mediate CpG-inducible host defenses during intraabdominal sepsis.

Sepsis remains a significant cause of mortality, despite active research and available therapeutic agents [1]. Recent evidence suggests that sepsis induces a significant state of immune suppression, as indicated by a reduction in Th1 cell responses and apoptosis of T, B, and dendritic cells (DCs) [2, 3]. Reversal of apoptosis and immune suppression in animal models correlates with improved outcomes from sepsis [4]. On the basis of these observations, positive immunomodulators may represent beneficial therapeutic agents for sepsis. One such immunomodulator is the Toll-like receptor (TLR) 9 ligand, an unmethylated dinucleotide CpG sequence motif (reviewed in [5]). The specific activity of the CpG ligand is determined by both the number of CpG motifs and the flanking nucleotide sequences [6]. CpG DNA is found in nonmammalian species, including viruses, protozoa, and bacteria, and induces a pathogen-associated molecular pattern response initiating host defense [6, 7].

CpG oligodeoxynucleotides (ODNs) were first utilized to treat intracellular pathogens through mechanisms that enhanced adaptive immunity, including DC maturation and up-regulation of major histocompatibility complex (MHC) class II proteins on DCs and macrophages [8]. CpG ODNs may induce proinflammatory cytokine production, including interferon (IFN)–γ, interleukin (IL)–6, and IL–12 [9]. CpG ODNs are also capable of enhancing innate immune effector-cell responses to extracellular pathogen infection. In both mouse and chicken models, CpG ODN pretreatment of the host reduced mortality and pathogen load after bacterial infection [10]. These studies demonstrated that CpG ODNs up-regulated local innate immune responses, including polymorphonuclear leukocyte (PMNL) and mononuclear cell infiltration and PMNL phagocytic function [10, 11]. The mechanism by which CpG ODNs altered innate immune function was not reported in these studies; however, in mice, CpG ODNs did not affect serum levels of IL–12, IL–18, or IL–10 [11].

The ability of CpG ODNs to affect both innate and adaptive immune function raises the possibility that components of each system may be involved in promoting beneficial outcomes from infection. The cyto-
kine IL-17 may represent one possible means of T cell regulation of innate immune responses. IL-17 is a member of a family of cytokines that is produced primarily by both T cells and PMNLs [12–14]. IL-17 receptor activation leads to synergistic expression of proinflammatory cytokines, including IL-1 and IL-6 [15, 16]. IL-17 expression elicits host protection in a mouse model of Klebsiella pneumonia and generates its effects on bacterial clearance early in the course of infection, suggesting an antigen-independent mechanism [17, 18]. In this model, IL-17 expression was induced by IL-23 and not IL-12, on the basis of the ability of p40-deficient—but not p35-deficient—mice to generate the IL-17 response [19]. In peritoneal infection models, IL-17 is required for both adhesion and abscess formation [20, 21]. The specific role of IL-17 in the host response to sepsis induced by cecal ligation and puncture (CLP) is unknown.

We sought to determine whether CpG ODNs might function as a protective immunomodulator when administered at the induction of sepsis, by use of a rodent CLP model. We also sought to determine whether CpG ODNs affect expression of IL-12 or IL-23 during CLP sepsis, by direct measurement of p35 and p40. Finally, we attempted to identify evidence linking IL-17 action to the beneficial effects of CpG ODNs during sepsis.

METHODS

ODNs. Phosphorothioate ODN sequences containing CpG motifs and control non-CpG ODN sequences were synthesized and purified by Qiagen. ODN sequences were as follows (CpG motifs are underlined): ODN 1826, TTCATGACG7CTTTGACGTT; and ODN 0x, TTCATGACG7CTTTGACGTT [22]. ODNs were analyzed for endotoxin contamination (>10 EU/mL) before use with an assay kit (BioWhittaker).

Antibodies. Polyclonal rabbit anti-mouse anti-IL-17 antibody was produced as described by Chung et al. [20].

CLP sepsis model. Specific pathogen-free, male Long-Evans rats were purchased from Charles River Labs. All procedures were conducted under an Institutional Animal Care and Use Committee–approved protocol. Anesthesia was induced with isofluorane, and oxygen was provided via a nose-cone mask. A 2-cm vertical midline incision was made in the lower abdomen and carried to the peritoneum by blunt dissection, and the cut end was ligated below the ileocecal valve by use of 4-0 vicryl sutures. The ligated cecum was punctured through-and-through by use of a 21-gauge needle and was compressed to express a small amount of fecal material, to ensure patency of the puncture sites. The cecum was returned to the abdominal cavity, the peritoneum and abdominal muscles were closed with 5-0 vicryl sutures, and the overlying skin was approximated with surgical staples. Rats received a 5-mL subcutaneous dose of normal saline for fluid resuscitation. Twenty minutes before closure of the skin, rats received a subcutaneous injection of 1.6 mg/kg of buprenorphine for analgesia. After surgery, rats received additional buprenorphine at 12-h intervals.

Bacterial load. At necropsy, liver and spleen were harvested, and the peritoneal cavity was lavaged with 5 mL of sterile normal saline. Equal wet tissue weights of liver and spleen were homogenized and briefly centrifuged to remove gross particulate matter. Serial log dilutions of peritoneal lavage fluid (PLF) and spleen and liver homogenates were applied to chocolate agar and blood agar plates and were incubated at 37°C for 48 h under anaerobic conditions and for 24 h under aerobic conditions, respectively. Plates were subsequently analyzed by colony counts, expressed as colony-forming units per milligram of wet tissue weight or colony-forming units per volume of PLF.

ELISA. ELISA analysis of rat IL-12 (p35) and IL-12/23 (p40) (Biosource) was performed according to the manufacturer’s specifications. Sample optical densities were read using a microtiter plate spectrophotometer (Spectramax 190; Molecular Devices), and data were analyzed using the Softmax Pro software package (Molecular Devices).

Tissue myeloperoxidase (MPO) activity. Measurement of tissue MPO activity was performed as described by Vakeva et al. [23]. Briefly, lung tissue was weighed and homogenized in 4 mL of buffer containing 0.25 mol/L sucrose and 0.1 mmol/L EDTA. Samples were centrifuged at 30,000 g at 4°C for 30 min. Pellets were subjected to freeze/thaw and sonication for 3 cycles. Tissue was homogenized in 0.5% hexadecyltrimethyl ammonium bromide in 500 mmol/L potassium phosphate buffer (pH, 6.0) and then was centrifuged at 12,500 g at 4°C for 15 min. Supernatants were collected and reacted with 0.167 mg/mL o-dianisidine dihydrochloride and 0.0005% H2O2 in 50 mmol/L potassium phosphate buffer (pH, 6.0). Changes in absorbance were measured on a microtiter plate spectrophotometer (Spectramax 190; Molecular Devices) at 460 nm for 10 min. The linear slope of the absorbance change was determined for each sample. One unit of MPO activity was defined as the quantity of MPO that hydrolyzed 1 μmol/L H2O2/min at 25°C. Final activity amounts were expressed as units per gram of wet tissue weight.

Histologic analysis of lung tissue. The right upper and middle lobes of the lung were harvested, and lung tissue was fixed in 10% formalin, paraffin embedded, sectioned, and stained with hematoxylin-eosin. Resultant sections were analyzed by an Nikon TE200 microscope, and representative digital photomicrographs were recorded at × magnification.

Apoptosis analysis. Apoptosis was analyzed using an annexin V/propidium iodide (PI) staining kit (R&D Systems). Thy-mocytes were isolated as described by Guo et al. [24]. Briefly, 106 cells/mL were incubated in the dark with fluorescein-conjugated annexin V for 5 min, followed by addition of PI to a final concentration of 1 μg/mL. Cells (a minimum of 20,000
per sample) were analyzed by flow cytometry using a Coulter EPICS XL cytometer (Becton Dickinson) and the CELLQuest software package (Becton Dickinson). Percentages of early apoptotic cells were determined by staining pattern (early apoptosis, annexin V+/PI−; late apoptosis, annexin V+/PI+).

Apoptotic thymocytes were also identified by TUNEL staining of thymus sections. Briefly, thymus tissue was fixed in 4% formalin overnight, embedded in paraffin, and sectioned. End-labeling of DNA fragments in tissue sections with fluorescein isothiocyanate–dUTP was performed according to the manufacturer’s specifications (Roche). Sections were also counterstained with an α-smooth-muscle actin–Cy3–conjugated antibody (Sigma). TO-PRO-3 was used to counterstain cell nuclei, as directed by the manufacturer (Molecular Probes). Slides were treated with ProlongAntifade Kit (Molecular Probes) and analyzed by confocal microscopy (Bio-Rad).

Statistical analysis. Statistical analysis was performed using analysis of variance (ANOVA), with the exception of survival analyses, which were performed using a log-rank test. The significance level was set at \( P < .05 \). Statistical analyses were performed using the Statview and JMP software packages (Abbaco/SAS Institute).

RESULTS

Prevention of mortality from CLP sepsis. Previous studies of the use of CpG ODNs for prevention of infection-induced complications have used pretreatment of the host animal before induction of the infection [11, 22]. To determine whether CpG ODNs could prevent mortality from sepsis, we induced CLP sepsis in Long-Evans rats, as described by Guo et al. [24]. We administered an intraperitoneal (ip) injection of 0.667 mg/kg ODN 1826 or ODN 0x at the conclusion of the CLP procedure. Mortality was assessed every 6–12 h for a total of 108 h. As is shown in figure 1, administration of ODN 1826 reduced 108-h mortality to 10%, versus 80% after administration of ODN 0x (\( P < .01 \), by log-rank test). There was no difference in cumulative 108-h mortality due to CLP sepsis in rats treated with saline (vehicle control; mortality, 25%) or 0.667 mg/kg ODN 0x (mortality, 20%) (\( P = .230 \), by log-rank test).

To determine the dose dependency of CpG ODN mortality protection in sepsis, we induced sepsis in Long-Evans rats by CLP and administered single doses of ODN 1826 in increasing concentrations (0.033, 0.167, and 0.667 mg/kg) by ip injection at the conclusion of CLP surgery. CpG ODN protection from sepsis mortality was dose dependent (figure 2), with maximal protection at a dose of 0.667 mg/kg (\( P = .005 \) and .01, for the 0.667 and 0.167 mg/kg groups, respectively, by log-rank test). No signs of toxicity were observed when the 0.667 mg/kg dose was used in sham-operated rats.

To test the clinical relevance of CpG therapy, we delayed the administration of CpG ODNs by 6 and 12 h after the induction of CLP sepsis. ODN 1826 was administered by ip injection, at a dose of 0.667 mg/kg, either 6 or 12 h after CLP. Figure 3 shows that delayed administration of ODN 1826 reduced mortality, compared with administration of control ODN 0x, when given 6 h after induction of sepsis (\( P < .001 \), by log-rank test). Administration of ODN 1826 12 h after CLP did not afford significant protection, compared with administration of control ODN 0x.

Reduction of early bacterial load in sepsis. To determine whether the survival benefit afforded by CpG ODNs was functionally related to pathogen load, we evaluated bacterial load 24 h after CLP. Sepsis was induced by CLP in Long-Evans rats

![Figure 1](image1.png)

**Figure 1.** Prevention of mortality from sepsis induced by cecal ligation and puncture, by use of CpG oligodeoxynucleotide (ODN) therapy. Survival in rats treated with ODN 1826 (\( \bullet \); black squares) vs. ODN 0x (\( \bigcirc \); white circles) is shown. \( P < .01 \) for comparison between the 2 groups, by log-rank test.

![Figure 2](image2.png)

**Figure 2.** Dose dependency of CpG oligodeoxynucleotide (ODN) protection in sepsis. Survival in rats (\( n = 5 \) rats/group) treated with ODN 1826 at 0.667 mg/kg (white squares), 0.167 mg/kg (white triangles), and 0.033 mg/kg (white circles) vs. ODN 0x (black diamonds) at 0.667 mg/kg is shown. \( P = .005 \) and .01 for the 0.667 and 0.167 mg/kg ODN 1826 groups, respectively, vs. the ODN 0x group, by log-rank test.
Figure 3. Protection in sepsis conferred by delayed CpG oligodeoxynucleotide (ODN) administration. Survival in rats ($n = 5$ rats/group) treated with ODN 1826 at 0.667 mg/kg 6 h (white squares) or 12 h (white circles) after cecal ligation and puncture vs. ODN 0x (black diamonds) at 0.667 mg/kg.
P < .001 for the 6-h delay ODN 1826 group vs. the ODN 0x group, by log-rank test.

Figure 4. Reduction in early bacterial load in sepsis, by use of CpG oligodeoxynucleotide (ODN) therapy. Peritoneal fluid, spleen, and liver were cultured under aerobic and anaerobic conditions 24 h after cecal ligation and puncture and treatment with ODN 1826 or ODN 0x.

$A$ rats/group. and .0012 (indicated by asterisks [*]) for aerobic and anaerobic cultures, respectively, by analysis of variance.

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treated with 0.667 mg/kg of either ODN 1826 or ODN 0x by ip injection at the end of CLP surgery. Peritoneal fluid, liver, and spleen were harvested 24 h after CLP and were cultured as described in the Methods section. After 24–48 h of growth, the number of colony-forming units was recorded for each plate; the averages are shown in figure 4. CpG ODN treatment significantly reduced both aerobic and anaerobic bacterial growth in peritoneal fluid 24 h after the induction of sepsis ($P < .0002$ and .0012, for aerobic and anaerobic cultures, respectively) (figure 4). Although there was a trend in the reduction of colony-forming units from spleen and liver with CpG ODN treatment (spleen aerobic cultures treated with ODN 0x vs. ODN 1826: mean ± SD, 21,314 ± 19,703 cfu vs. 0 cfu; spleen anaerobic cultures treated with ODN 0x vs. ODN 1826: 3400 ± 2227 cfu vs. 7 ± 7 cfu; liver aerobic cultures treated with ODN 0x vs. ODN 1826: 3664 ± 2858 cfu vs. 0 cfu; liver anaerobic cultures treated with ODN 0x vs. ODN 1826: 106 ± 58 cfu vs. 1000 ± 1000 cfu), the differences were not statistically significant (figure 4).

Increase in expression of the p40 component of IL-23/IL-12 but not of the p35 component of IL-12. Previous studies have shown that IL-12 expression is enhanced by CpG ODN stimulation of DCs [22, 25]. IL-12 is a heterodimeric protein composed of both p40 and p35 subunits (reviewed in [26]). The p40 component of IL-12 has been identified as a component of IL-23, which is composed of p40 and p19 subunits [27]. To determine whether CpG ODN treatment affected IL-23 and IL-12 expression during sepsis, we analyzed peritoneal fluid and plasma by use of ELISAs specific for p40 or p35. Long-Evans rats were treated with 0.667 mg/kg of either ODN 1826 or ODN 0x by ip injection at the end of CLP surgery. Twelve and 24 h after CLP, peritoneal fluid and plasma were harvested, and expression levels of both p40 and p35 were individually determined. Figure 5 shows that ODN 1826 significantly increased p40 expression levels in peritoneal fluid and plasma 12 h after CLP. Twelve hours after CLP, p40 levels were significantly elevated in the plasma of ODN 1826–treated rats (figure 5A). There was a trend for greater expression of p40 in peritoneal fluid from ODN 1826–treated rats than from ODN 0x–treated controls at 24 h after CLP; however, this difference was not significant (figure 5B). We did not observe measurable expression of p35 in peritoneal fluid or plasma from either ODN 1286–treated or ODN 0x–treated rats 24 h after CLP. Indirectly, these data suggest that at least some of the CpG ODN biological effects may be mediated through IL-23 rather than IL-12.

Dependency of protection from mortality in sepsis on IL-17. The cytokine IL-17 is induced by IL-23 during pulmonary infection and also mediates abscess formation in the peritoneal cavity [19, 21]. On the basis of published data and our observations of increased p40 expression, we wished to test whether the protective CpG ODN mechanism of action was dependent on induction of IL-17. Long-Evans rats underwent CLP and were treated with 0.667 mg/kg of ODN 1826 by ip injection at the end of CLP surgery. At the end of CLP surgery, rats were also randomized to receive an ip injection of either purified IgG anti–IL-17 antibody (200 μg/rat) or IgG control antibody (200 μg/rat). A second dose of anti–IL-17 antibody or corresponding IgG control antibody was administered by ip injection 12 h after the initial dose, to ensure adequate reduction of IL-17 during the first 24 h of experimental sepsis. Rats were observed for mortality every 6–12 h during a 108-h period. As is shown in figure 6, administration of anti–IL-17 antibody inhib-
Increased expression of p40 but not p35 during sepsis, after treatment with CpG oligodeoxynucleotides (ODNs). A, p40 levels in plasma at 12 and 24 h after cecal ligation and puncture (CLP) and treatment with ODN 1826 or ODN 0x. Asterisks (*) indicate, by analysis of variance (ANOVA), P < .01. B, p40 levels in peritoneal fluid 12 and 24 h after CLP and treatment with ODN 1826 or ODN 0x. The asterisk (*) indicates P < .004, by ANOVA.

Reduction in sepsis-induced lung injury. Sepsis induces multiple-organ damage at sites distant from the primary infection. To determine whether CpG ODNs had a beneficial effect on organ systems distinct from the peritoneal compartment during sepsis, we chose to evaluate inflammatory changes in lung tissue after CLP. Rats underwent sham surgery or CLP surgery, followed by treatment with 0.667 mg/kg of either ODN 1826 or ODN 0x by ip injection. Twenty-four hours after CLP, lung tissue was harvested and processed for histologic assessment and measurement of MPO activity. CLP induced a 28-fold increase in total lung MPO activity after 24 h, compared with that in sham-operated controls (P < .002; table 1). ODN 1826 treatment reduced total lung MPO activity to control levels; there was no statistical difference in MPO activity between these groups (P = .32). Histologic analysis showed interstitial infiltration, predominantly of mononuclear cells, in lungs from CLP-septic rats treated with ODN 0x, compared with lungs from sham-operated controls (figure 7). Cellular infiltration was reduced in CLP-septic rats treated with ODN 1826, compared with those treated with ODN 0x (figure 7).

Reduction in sepsis-induced thymocyte apoptosis. Apoptosis of lymphocytes represents another marker for severity of sepsis and correlates with mortality [28]. CpG ODNs may prevent DC apoptosis in vitro; however, there are no studies describing CpG ODN effects on apoptosis prevention on the basis of an in vivo sepsis model [29]. We wished to determine whether CpG ODN therapy reduced sepsis-induced lymphocyte apoptosis. Long-Evans rats (n = 5 rats/group) were subjected to CLP and treated with 0.667 mg/kg of either ODN 1826 or ODN 0x by ip injection. Twenty-four hours after surgery, thymocytes were prepared from individual rats and were analyzed by fluorescence-activated cell sorting for apoptosis, by use of annexin V/PI staining. Treatment with ODN 1826 significantly reduced the number of late-apoptotic cells, measured as the mean ± SD percentage of annexin V+/PI–staining cells (ODN 1826, 9.6% ± 3.3%; ODN 0x, 26.7% ± 4.2%; n = 5; P = .004). Representative dot plots are shown in figure 8A and 8B. There was no significant difference in early apoptotic annexin V+/PI–staining cells (ODN 1826, 32.3% ± 0.3%; ODN 0x, 35.2% ± 6.7%; n = 5; P = .62). Inhibition of apoptosis by ODN 1826 but not ODN 0x was also demonstrated by TUNEL staining of thymus tissue (figure 8C and 8D). We did not observe any differences in splenocyte apoptosis after treatment with ODN 1826 versus ODN 0x, with respect to annexin V/PI staining (data not shown).
Table 1. Reduction in total lung myeloperoxidase (MPO) activity in sepsis induced by cecal ligation and puncture (CLP), by use of CpG oligodeoxynucleotide (ODN) therapy.

<table>
<thead>
<tr>
<th>Group</th>
<th>MPO concentration, mean ± SD, U/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>0.041 ± 0.024</td>
</tr>
<tr>
<td>CLP + treatment with ODN 0x</td>
<td>1.149 ± 0.146a</td>
</tr>
<tr>
<td>CLP + treatment with ODN 1826</td>
<td>0.324 ± 0.190b</td>
</tr>
</tbody>
</table>

**NOTE.** n = 5 rats/group.

- *P* = .002 vs. sham-operated group.
- *P* = .003 vs. ODN 0x group; *P* = .32 vs. sham-operated group.

**DISCUSSION**

Prevention of immune suppression may lead to beneficial outcomes during sepsis and may represent a novel therapeutic intervention point. CpG ODNs have been shown to enhance immune function during bacterial, viral, and parasitic infections [5]. The mechanism of action of CpG ODNs in sepsis may be related to the enhancement of innate and adaptive immune responses, depending on the pathogen model studied. Our results are in opposition to those suggesting that CpG ODN therapy may worsen outcome in shock [30, 31]. Studies of macrophage activation by CpG DNA and the cloning of TLR9 have demonstrated that TLR9 activation by CpG ODNs enhanced lethality in mice sensitized with d-galactosamine. The use of d-galactosamine may not accurately recreate an environment similar to sepsis resulting from pathogen infection. Our findings correlate with those showing CpG-mediated protection from morbidity and mortality after pathogenic infection [10, 11].

The ability of CpG ODNs to provide protection in sepsis at the time of CLP and after CLP may be related to the specific model of sepsis and the species used for the study. Our study employed a rat model rather than the previously studied mouse and chicken extracellular infection models [10, 11]. It is possible that species differences could account for the enhanced protection conferred by CpG ODNs in our study, compared with that in previous studies [10, 11]. Also, the use of CpG ODNs has not yet been reported in conjunction with the CLP model. Previous studies of CpG ODN protection from extracellular infections have employed models of cellulitis and peritoneal infection via cecal ascending stent peritonitis (CASP) [10, 11]. The CASP model is significantly different from the CLP model. The CASP procedure utilizes placement of a stent that allows communication between the cecal contents and the peritoneal cavity. The CASP model induces peritoneal infection via the leakage of fecal material. The CLP model utilizes ligation of the cecum, with the cecal perforation distal to the ligated portion of the colon. The ligated cecum itself may become necrotic and generate an inflammatory response in addition to that generated by the leaking fecal material. This is the mechanism postulated to explain the observed increase in mortality that is proportional to the amount of cecum ligated, when the CLP model is used [32]. Since the CASP model does not have this secondary inflammatory insult due to the ligated cecum, it is...
likely a less aggressive model of sepsis. Because of the significant differences in the models used for the study of CpG ODN protection, it is difficult to compare their results directly, since the mechanism of protection may differ slightly in each model system studied.

Prior studies have suggested that the effects of CpG pretreatment were due to the enhancement of innate immune effector-cell function [11]. The mechanism accounting for functional up-regulation of innate immune cells by CpG ODNs is unclear. CpG ODNs could have direct effects on innate immune cells; however, the TLR9 receptor is most prominently expressed on DCs and macrophages in the murine system [33]. Clearly, differences exist between human and murine cells in the expression of TLR9. Current understanding of TLR9 expression comes largely from studies of isolated, quiescent cells. TLR9 expression may be induced after cell stimulation with exogenous agents [34]. Expression of TLR9 is not completely characterized in humans and mice during sepsis. It is unknown whether TLR9 may be induced on cell populations during the development of the sepsis response. It is possible that TLR9 could be induced on various cell types in a compartmentalized manner during the development of sepsis, since there is dynamic modification of the immune response during this period. It was recently shown that human PMNLs may express TLR9 and that PMNLs may differentiate, depending on host circumstances, to respond to TLR9 ligands [34, 35, 36]. Expression of TLR9 on rodent PMNLs has not been documented, although it is possible that improved outcome in sepsis in our model system could be due to enhanced innate immune function via stimulation of PMNL TLR9, if present. Therefore, it is difficult to extrapolate the utility of CpG ODNs to the treatment of human sepsis on the basis of current knowledge of restricted TLR9 expression. Such extrapolation will require characterization of TLR9 expression and CpG ODN effects during the development of sepsis in each experimental model system (CLP vs. CASP), as well as in each source of human sepsis (pneumonia vs. ip infection).
Since our findings suggested that CpG ODNs enhanced expression of IL-23 p40, we sought to determine whether IL-17 could possibly represent a cytokine bridge between the TLR9-bearing cell population and innate immune effector cells responsive to IL-17. The increase in expression of p40 suggests an initial mechanism of interaction between macrophages/DCs and T cells or PMNLs during early bacterial sepsis. It was recently shown that IL-23 is responsible for increases in T cell secretion of IL-17 [19]. On the basis of this finding, it is possible that CpG ODNs activate T cells or PMNLs via IL-23 produced by macrophages or DCs during early sepsis. Subsequent IL-17 release from T cells or PMNLs might then feed back on cells involved with the innate immune response, producing a complete communication network loop. The amplification of this T cell–innate immune cell network represents a potential novel point of therapeutic intervention in sepsis by use of CpG ODNs. However, since we were unable to measure p19 protein expression directly, it is possible that our observed outcomes result from the activity of p40 homodimers rather than of the IL-23 heterodimer p19/p40 [37].

Our results suggest that IL-17 expression or function may be required for the full effect of CpG ODN therapy and suggest a possible role for IL-17 as a regulator of the host response to bacterial sepsis. IL-17 is known to play a role in the formation of ip abscesses and adhesion formation during experimental infection [21]. Results of another study suggest that inhibition of IL-12 may lead to reduced abscess formation during CLP [38]. These observations suggest a hypothesis whereby maintenance of adequate IL-12/23 p40 and IL-17 expression or function is required for survival from CLP sepsis. Our model of CpG ODN therapy highlights the potential link between IL-12/23 p40 expression and IL-17 function in the containment of peritoneal bacterial infection.

We did not detect differences in IL-17 levels in peritoneal fluid in CpG and non-CpG ODN–treated rats (data not shown). Results of a prior study of the role of IL-17 in the formation of peritoneal abscesses suggest that it may be produced and act in a localized manner [21]. Although our findings implicate IL-17 in the CpG ODN protective mechanism, it is possible that this is not a result of altered IL-17 levels. It is possible that CpG ODNs induce IL-17 receptor expression or function, which would explain the CpG ODN–mediated IL-17–dependent effect indirectly. We did not address the source of IL-17 involved in the protective CpG mechanism during sepsis in our model. The predominant source and role of IL-17 during intraabdominal infections remain unknown. Previous work has suggested that IL-17 derived from CD4+ T cells is required for effective abscess formation in a murine model of bacterial implantation [21]. The specific role of PMNL-derived IL-17 in abscess formation is undefined. However, since PMNLs are recruited to the peritoneal cavity and infection site, it is possible that they also contribute local IL-17 to the inflammatory process.

Our results suggest that CpG ODN therapy for sepsis reduces lung inflammation. The reduction in MPO activity was correlated with a reduction in mononuclear cell interstitial infiltration after CpG ODN administration. These findings are consistent with published findings that macrophages represent a significant source of MPO and also are required for PMNL recruitment to lung [39, 40]. The ability of CpG ODNs to prevent sepsis-induced lung inflammation has not been described. Previous studies have shown that CpG ODNs may induce lung inflammation if they are directly applied to lung via the tracheal route [41]. These studies did not evaluate the effect of CpG ODNs on lung infection or the effect of indirect CpG ODN administration on lung inflammation. Direct use of CpG ODNs during lung infection has not been documented.

Our model does not allow determination of whether CpG ODN therapy directly or indirectly reduces lung inflammation after CLP. Regardless, our results suggest that CpG ODN administration at sites distant from lung does not promote generalized inflammation during the development of sepsis. Our findings and the documented ability of CpG ODNs to prevent asthma-induced lung inflammation suggest that the context of CpG ODN administration is an important contributor to the final physiologic outcome [42].

Prior studies have suggested that lymphocyte and DC apoptosis are detrimental sequelae of sepsis [3, 43]. CpG ODNs may prevent or induce apoptosis, depending on the ODN structure [29, 44]. ODNs containing a 5′ CpG motif may induce apoptosis through a mitochondrial-dependent mechanism in isolated T leukemia cells [44]. However, ODNs containing a central CpG motif may prevent DC and B cell apoptosis directly in vitro [29]. The function of CpG ODNs in vivo is unknown. Our data suggest that the therapeutic application of CpG ODNs can inhibit late apoptosis of thymocytes induced by CLP. It is not clear from our experiments whether CpG ODNs inhibited apoptosis directly or indirectly. Direct inhibition of apoptosis by CpG ODNs has been described in vitro [29, 45]. However, indirect apoptosis is also possible in vivo, through the prevention of sepsis progression in the host. Future studies may clarify the action of CpG ODNs in preventing apoptosis in vivo during the development of sepsis.

References


