Interleukin 10 Protects Mice from Lethal Endotoxemia
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Summary
Interleukin 10 (IL-10) decreases production of IL-1, IL-6, and tumor necrosis factor α (TNF-α) in vitro, and neutralization of IL-10 in mice leads to elevation of the same monokines. We test here whether this monokine-suppressing property of IL-10 confers on it the capacity to protect mice from lipopolysaccharide-induced shock, a monokine-mediated inflammatory reaction. A single injection of 0.5–1 μg of recombinant murine IL-10 reproducibly protected BALB/c mice from a lethal intraperitoneal injection of endotoxin. This result was obtained whether the IL-10 was administered concurrently with, or 30 min after the injection of endotoxin. The protective effect of IL-10 was reversed by prior injection of neutralizing anti-IL-10 antibodies, and correlated with a substantial decrease in endotoxin-induced TNF-α release. These data implicate IL-10 as a candidate for treatment of bacterial sepsis, and more generally as an effective antiinflammatory reagent.

Severe bacterial infections can result in profound physiological changes including hypotension, fever, tissue necrosis, widespread organ dysfunction, and ultimately death. In the case of gram-negative bacteria, this toxicity is due to endotoxin, a LPS component of the bacterial cell wall (1, 2). Indeed, injection of appropriate doses of LPS into rabbits, mice, and other animals changes that are typical of the septic shock syndrome, thus yielding a simple animal model of this inflammatory reaction. Endotoxin-induced toxicity appears to be due to the release of TNF-α and/or IL-1 from endotoxin-stimulated macrophages/monocytes, since animals can be protected from bacterial and endotoxin-induced shock by neutralization of these monokines, using either mAbs or a physiological IL-1 antagonist termed IL-1ra (3, 4).

IL-10 is a 35-kD protein produced as a result of immune activation by subpopulations of helper T cells (5, 6), B cells (7, 8), and macrophage/monocytes (9, 10). Its numerous in vitro properties (for reviews see references 11 and 12) include suppression of IFN-γ production by helper T cells and NK cells (5, 13), growth costimulation of thymocytes, mast cells, and B cells (14–17), and suppression of monokine production (9, 10, 18, 19). With respect to the latter property, IL-10 profoundly suppresses the induced production of TNF-α, IL-1α, IL-1β, IL-6, IL-8, and GM-CSF by human monocytes (9) and mouse peritoneal macrophages (10). In contrast, IL-10 has no effect on constitutive expression of TGF-β by monocytes (9) and actually upregulates monocyte production of the IL-1ra (20). These in vitro data are supported by in vivo experiments showing that neutralization of IL-10 using specific mAbs leads to elevated levels of circulating TNF-α and IL-6 in mice (11). We test here whether the ability of IL-10 to suppress production of TNF-α and IL-1 together with its ability to increase IL-1ra, renders this cytokine capable of protecting mice against endotoxin-induced shock.

Materials and Methods
Mice. 8-wk-old BALB/c female mice were obtained from Simonsen Laboratories (Gilroy, CA). Animals were kept in the DNAX Animal Facility for a consistent 2.5 d before experimentation to help minimize animal-to-animal variation.

Reagents. LPS from Escherichia coli serotype 0111:B4 was purchased from Sigma Immunochemicals (St. Louis, MO). Two separate preparations (designated batch No. 1 and batch No. 2) prepared in an identical manner were used throughout the entire study. Recombinant murine IL-10 was expressed in E. coli and purified to homogeneity and high sp act (~1.75 x 10⁶ U/mg) after refolding using hydrophobic and ion exchange chromatography. The protein concentration in the purified preparations was determined by the extinction coefficient of the protein (1 mg/ml = 0.36 A₂₅₀). This material contained <0.10 eu/mg protein of endotoxin, and remained stable at 4°C for at least 6 mo. The specific activity of murine IL-10 was evaluated in the cytokine synthesis inhibition assay (5). Recombinant murine IL-10 was expressed in E. coli and purified to homogeneity and high sp act (<1.75 x 10⁶ U/mg) after refolding using hydrophobic and ion exchange chromatography. The protein concentration in the purified preparations was determined by the extinction coefficient of the protein (1 mg/ml = 0.36 A₂₅₀). This material contained <0.10 eu/mg protein of endotoxin, and remained stable at 4°C for at least 6 mo. The specific activity of murine IL-10 was evaluated in the cytokine synthesis inhibition assay (5). Recombinant IL-10 was diluted in PBS containing 0.1% BSA, and administered to mice at various concentrations in a total volume of 100 μl. Neutralizing antibody experiments utilized the 2A5 rat IgG₁ anti-mouse IL-10 mAb (21), or an isotype control antibody designated GL113.

Endotoxin-induced Shock. Mice were injected intraperitoneally with 100 μl vol containing doses of endotoxin ranging from 250 to 425 μg. The dose-response curves of animal survival versus endotoxin dose using either of two different preparations of endotoxin are shown in Table 1. From these data, the LD₉₀ selected for LPS batch No. 1 and LPS batch No. 2 was 350 μg/mouse and 400 μg/mouse, respectively.
**TNF-α Assay.** Serum levels of TNF-α were evaluated using a cytokine-specific ELISA, commercially available from Endogen, Inc. (Boston, MA).

**Results and Discussion**

To evaluate the effect of IL-10 on lethal endotoxemia in mice, groups of 20 BALB/c mice were injected intraperitoneally with 100 μl containing an LD₅₀ of LPS (between 350 and 400 μg, depending on the batch of endotoxin), together with an additional 100 μl containing either PBS or varying amounts of recombinant murine IL-10. In seven independent experiments of this type, mice were completely protected from death resulting from LPS-induced shock when either 0.5, 1.0, or 10 μg of IL-10 was administered to the animal concurrently with the LPS (Fig. 1 and Table 1). In most of these experiments, a substantial proportion of mice were protected after receiving 0.1 or 0.05 μg of IL-10 at the time of LPS administration (Fig. 1 and Table 1). IL-10–mediated protection of mice from lethal endotoxemia could be blocked by prior adminis-

![Figure 1](image1.png)

**Figure 1.** IL-10 protects mice from lethal endotoxemia. Six groups of 20 BALB/c mice were injected intraperitoneally with 350 μg LPS, together with 100 μl PBS (□) or an equivalent volume containing the following doses of purified recombinant murine IL-10: 0.05 μg (■), 0.1 μg (○), 0.5 μg (▲), 1.0 μg (●), or 10 μg (▲). Death was monitored over the following 7 d. Similar results were obtained in six additional experiments (refer to Table 1).

<table>
<thead>
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<th>LPS/mouse</th>
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**Table 1.** Survival of BALB/c Mice Injected Intraperitoneally with Different Amounts of Endotoxin

![Figure 2](image2.png)

**Figure 2.** Anti-IL-10 antibodies neutralize the ability of IL-10 to protect mice from lethal endotoxemia. Groups of 20 BALB/c mice received either 1 mg of 2A5 anti-IL-10 antibody or 1 mg of GL13 isotype control antibody intraperitoneally 1 h before LPS administration. Mice were then injected intraperitoneally with 400 μg LPS either alone, or concurrently with varying doses of IL-10, as described in the Fig. 1 legend. Similar results were obtained in two separate experiments.

![Figure 3](image3.png)

**Figure 3.** IL-10 protects mice from lethal endotoxemia when administered 30 min after LPS injection. Groups of 20 BALB/c mice received 350 μg LPS intraperitoneally at time 0, and 1.0 μg IL-10 intraperitoneally at either time 0, or 0.5, 1, 2, or 5 h after LPS injection. Animal survival was monitored over the following 7 d. 20 control mice receiving 350 μg LPS intraperitoneally in the absence of IL-10 all died in this experiment. Similar results were obtained in three separate experiments.
IL-10 suppresses TNF-α release after LPS administration. Groups of 20 BALB/c mice were injected intraperitoneally with either 350 μg LPS (O), or 350 μg LPS concurrently with 1 μg of IL-10 (●). Sera were collected 1, 2, 3, and 4 h later, and assayed for TNF-α content by ELISA. Each circle represents an individual mouse. Similar results were obtained in three separate experiments.

Figure 4. IL-10 suppresses TNF-α release after LPS administration. Groups of 20 BALB/c mice were injected intraperitoneally with either 350 μg LPS (O), or 350 μg LPS concurrently with 1 μg of IL-10 (●). Sera were collected 1, 2, 3, and 4 h later, and assayed for TNF-α content by ELISA. Each circle represents an individual mouse. Similar results were obtained in three separate experiments.

Table 2. IL-10 Protects Mice from Death Resulting from Endotoxin-induced Shock

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<tr>
<th>IL-10/μg</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
<th>Expt. 3</th>
<th>Expt. 4</th>
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* Mice in expts. 1–6 received 350 μg i.p. LPS batch no. 1.

† Mice in expts. 6 and 7 received 400 μg i.p. LPS batch no. 2.
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References


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