Brief Definitive Report

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 produces 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 × 10^6 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 A_{280}). 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.

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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 LD_{90} 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-

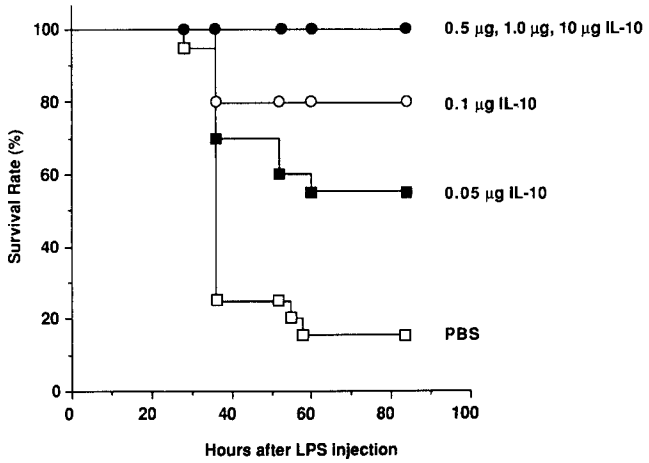

Figure 1. IL-10 protects mice from lethal endotoxemia. Six groups of 20 BALB/c mice were injected intraperitoneally with 350 μg LPS, together with 100 μl PBS (□) or an equivalent volume containing the following doses of purified recombinant murine IL-10: 0.05 μg (■), 0.1 μg (○), 0.5 μg (●), 1.0 μg (●),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

Table 1. *Survival of BALB/c Mice Injected Intraperitoneally with Different Amounts of Endotoxin*

	No. survivors/total mice	
LPS/mouse	LPS batch no. 1	LPS batch no. 2
μg		
250	14/30	14/20
275	9/30	ND
300	9/30	5/20
325	5/30	ND
350	3/30	5/20
375	4/30	5/20
400	3/30	1/20
425	0	2/20

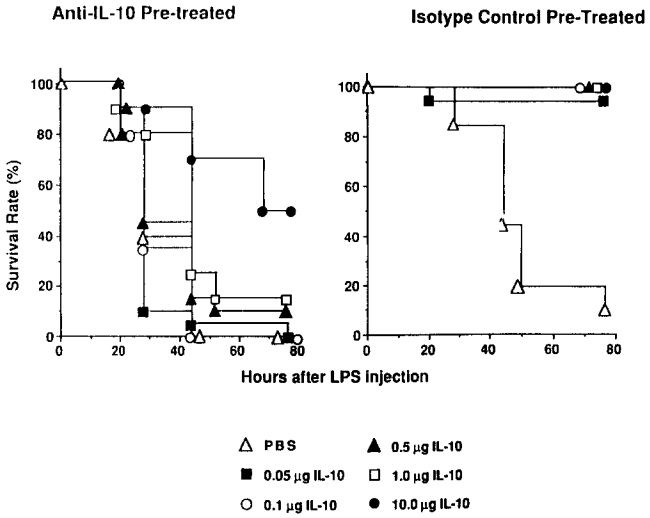

Figure 2. Anti-IL-10 antibodies neutralize the ability of IL-10 to protect mice from lethal endotoxemia. Groups of 20 BALB/c mice received either 1 mg of 2A5 anti-IL-10 antibody or 1 mg of GL113 isotype control antibody intraperitoneally 1 h before LPS administration. Mice were then injected intraperitoneally 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.

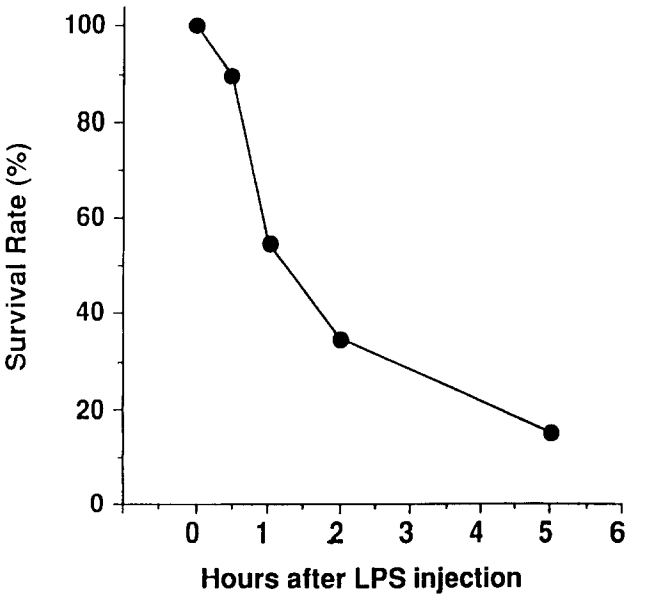

Figure 3. IL-10 protects mice from lethal endotoxemia when administered 30 min after LPS injection. Groups of 20 BALB/c mice received 350 μg LPS intraperitoneally at time 0, and 1.0 μg rIL-10 intraperitoneally at either time 0, or 0.5, 1, 2, or 5 h after LPS injection. Animal survival was monitored over 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.

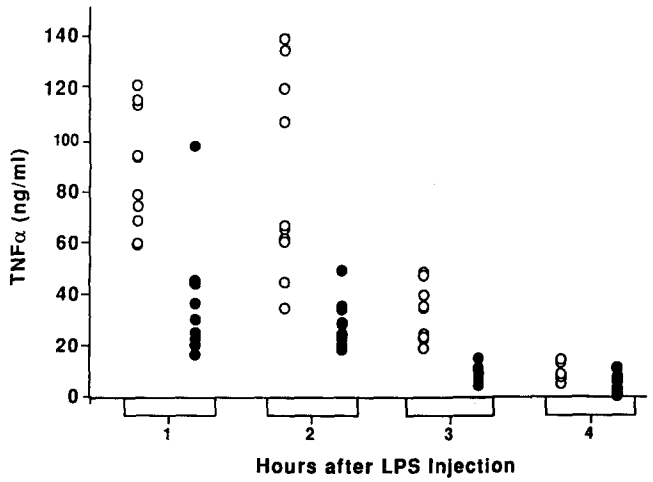

Figure 4. IL-10 suppresses TNF-α release after LPS administration. Groups of 20 BALB/c mice were injected intraperitoneally with either 350 μg LPS (○), or 350 μg LPS concurrently with 1 μg of IL-10 (●). Sera were collected 1, 2, 3, and 4 h later, and assayed for TNF-α content by ELISA. Each circle represents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

tration of neutralizing anti-IL-10 antibodies, but not by an isotype control antibody (Fig. 2), confirming the specificity of this effect. A kinetics study revealed that IL-10–mediated protection of mice from lethal endotoxemia was achieved even if the IL-10 was administered 30 min after the LPS injection (Fig. 3). However, further delays in IL-10 administration substantially reduced protection, and no protection was observed when IL-10 was administered 5 h after the LPS injection (Fig. 3).

Lethal endotoxemia is an undesirable monokine-mediated inflammatory reaction (3, 4). Since IL-10 has been shown to suppress monokine production by activated macrophages and monocytes in vitro (9, 10, 18, 19), we considered the possibility that the above IL-10–mediated protection reflected a suppression of monokine production in the endotoxin-induced response. Indeed, sera collected 1, 2, 3, and 4 h after LPS ± IL-10 injection indicated a substantial reduction in circulating TNF-α levels in animals receiving LPS plus IL-10 compared with animals receiving LPS alone (Fig. 4). Since anti-TNF antibodies similarly protect mice from lethal endotoxemia (3), it is likely that IL-10-induced suppression of TNF-α at least contributes to the protection this cytokine provides against lethal endotoxemia. Other properties of IL-10 may also contribute to this protection. In particular, in vitro studies have indicated that IL-10 also suppresses IL-1 production, and upregulates IL-1ra production, by activated macrophages (9, 10, 21). Both of these consequences will presumably also contribute to protection of mice from lethal endotoxemia in light of previously published reports showing the protective effects of direct IL-1ra administration in this type of model (4).

In summary, our data indicate that IL-10 is highly effective at protecting mice from lethal endotoxemia, a finding which suggests that IL-10 may be an important candidate for treatment of bacterial sepsis. Whereas numerous other reagents e.g., antibodies to TNF-α (3) or endotoxin (22–25), as well as the IL-1ra (4), are currently in clinical trials for treatment of bacterial sepsis, most of these need to be given before endotoxemia induction in animal model experiments in order to obtain optimal protection. One exception to this, the IL-1ra, is effective when administered at the time of endotoxemia induction in animal model experiments, but must be administered in quantities large enough to block all endogenous IL-1 receptors (4). Since pharmacological doses of IL-10 produce an array of effects on macrophage/monocyte function that potentially will all contribute to protection from lethal endotoxemia, it will be interesting to compare the efficacy of this cytokine versus other current candidates in the treatment of bacterial sepsis.

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

	No. survivors/total mice							
IL-10/mouse	Expt. 1	2	3	4	5	6	7	Total
μg								
0	4/20*	1/20	2/20	2/20	3/20	2.20‡	1/20	15/140
0.05	19/20	20/20	19/20	7/20	11/20	11/20	4/20	91/140
0.1	20/20	20/20	20/20	15/20	16/20	16/20	13/20	120/140
0.5	20/20	20/20	20/20	19/20	20/20	20/20	19/20	138/140
1.0	20/20	20/20	20/20	20/20	20/20	20/20	20/20	140/140
10	20/20	20/20	20/20	20/20	20/20	20/20	20/20	140/140

* 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

1. Westphal, O. 1975 Bacterial endotoxins. *Int. Arch. Allergy Appl. Immunol.* 49:1.
2. Gilbert, R.P. 1960. Mechanisms of the hemodynamic effects of endotoxin. *Physiol.* 40:245.
3. Beutler, B., I.W. Milsark, and A.C. Cerami. 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science (Wash. DC).* 229:869.
4. Alexander, H.R., G.M. Doherty, C.M. Buresh, D.J. Venzon, and J.A. Norton. 1991. A recombinant human receptor antagonist to interleukin 1 improves survival after lethal endotoxemia in mice. *J. Exp. Med.* 173:1029.
5. Fiorentino, D.F., M.W. Bond, and T.R. Mosmann. 1989. Two types of mouse T helper cell: IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170:2081.
6. Vieira, P., R. de Waal-Malefyt, M.-W. Dang, K.E. Johnson, R. Kastelein, D.F. Fiorentino, J.E. de Vries, M.-G. Roncarolo, T.R. Mosmann, and K.W. Moore. 1991. Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. *Proc. Natl. Acad. Sci. USA.* 88:1172.
7. O'Garra, A., G. Stapleton, V. Dhar, M. Pearce, J. Schumacher, H. Rugo, D. Barbis, A. Stall, J. Cupp, K. Moore, P. Vieira, T. Mosmann, A. Whitmore, L. Arnold, G. Haughton, and M. Howard. 1990. Production of cytokines by mouse B cells: B lymphomas and normal B cells produce interleukin 10. *Int. Immunol.* 2:821.
8. O'Garra, A., R. Chang, R. Hastings, N. Go, G. Haughton, and M. Howard. 1992. Ly1 B (B-1) cells are the main source of B-cell derived IL-10. *Eur. J. Immunol.* 22:711.
9. de Waal Malefyt, R., J. Abrams, B. Bennett, C. Figdor, and J.E. de Vries. 1991. IL-10 inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174:1209.
10. Fiorentino, D.F., A. Zlotnik, T.R. Mosmann, M. Howard, and A. O'Garra. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147:3815.
11. Howard, M., A. O'Garra, H. Ishida, R. de Waal Malefyt, and J. de Vries. 1992. Biological properties of interleukin 10. *J. Clin. Immunol.* 12:239.
12. Howard, M., and A. O'Garra. 1992. Biological properties of interleukin 10. *Immunol. Today.* 12:198.
13. Hsu, D.-H., K.W. Moore, and H. Spits. 1992. Differential effects of interleukin-4 and -10 on interleukin-2–induced interferon-γ synthesis and lymphokine-activated killer activity. *Int. Immunol.* 4:563.
14. MacNeil, I.A., T. Suda, K.W. Moore, T.R. Mosmann, and A. Zlotnik. 1990. IL-10, a novel growth cofactor for mature and immature T cells. *J. Immunol.* 145:4167.
15. Thompson-Snipes, L., V. Dhar, M.W. Bond, T.R. Mosmann, K.W. Moore, and D.M. Rennick. 1991. Interleukin 10: a novel stimulatory factor for mast cells and their progenitors. *J. Exp. Med.* 173:507.
16. Go, N.F., B.E. Castle, R. Barrett, R. Kastelein, W. Dang, T.R. Mosmann, K.W. Moore, and M. Howard. 1990. Interleukin 10, a novel B cell stimulatory factor: unresponsiveness of X chromosome-linked Immunodeficiency B cells. *J. Exp. Med.* 172:1625.
17. Rousset, F., E. Garcia, T. Defrance, C. Peronne, N. Vezzio, D.H. Hsu, R. Kastelein, K.W. Moore, and J. Banchereau. 1992. IL-10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 89:1890.
18. Bogdan, C., Y. Vodovotz, and C. Nathan. 1991. Macrophage deactivation by interleukin-10. *J. Exp. Med.* 174:1549.
19. Ralph, P., I. Nakoinz, A. Sampson-Johannes, S. Fong, D. Lowe, H.-Y. Min, and L. Lin. 1992. IL-10, T lymphocyte inhibitor of human blood cell production of IL-1 and tumor necrosis factor. *J. Immunol.* 148:808.
20. de Waal Malefyt, R., H. Yssel, M.-G. Roncarolo, H. Spits, and J. de Vries. 1992. Interleukin 10. *Curr. Opin. Immunol.* 4:314.
21. Abrams, J.S., M.-G. Roncarolo, H. Yssel, U. Andersson, G.J. Gleich, and J.E. Silver. 1992. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol. Rev.* 127:5.
22. Ziegler, E.J., J.A. McCutchan, J. Fierer, M.P. Glauser, C. Sadoff, H. Douglas, and A.I. Braude. 1982. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N. Engl. J. Med.* 307:1225.
23. Baumgartner, J.D., M.P. Glauser, J.A. McCutchan, J. Ziegler, G. van Melle, M.R. Klauber, M. Vogt, E. Muehlen, R. Lüthy, R. Chiolero, and S. Geroulanos. 1985. Prevention of gram-negative shock and death in surgical patients by prophylactic antibody to endotoxin core glycolipid. *Lancet.* ii:59.
24. Teng, N.N.H., H.S. Kaplan, and J.M. Hebert. 1985. Protection against Gram-negative bacteremia and endotoxemia with human monoclonal IgM antibodies. *Proc. Natl. Acad. Sci. USA.* 82:1790.
25. Baumgartner, J.D., D. Heumann, J. Gerain, P. Weinbreck, G.E. Grau, and M.P. Glauser. 1990. Association between protective efficacy of anti-lipopolysaccharide (LPS) antibodies and suppression of LPS-induced tumor necrosis factor alpha and interleukin 6. Comparison of O side chain-specific antibodies with core LPS antibodies. *J. Exp. Med.* 171:889.