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An Interleukin-1 Receptor Antagonist Blocks Lipopolysaccharide-Induced Colony-Stimulating Factor Production and Early Endotoxin Tolerance

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In this report, administration of a recombinant interleukin-1 receptor antagonist protein to mice was found to inhibit induction of colony-stimulating factor as well as induction of early endotoxin tolerance by lipopolysaccharide. These findings provide direct evidence that interleukin-1 is an intermediate in these two lipopolysaccharide-induced phenomena.

Lipopolysaccharide (LPS) is located exclusively in the outer membrane of gram-negative bacteria and is the moiety responsible for the vast majority of biological activities associated with gram-negative bacterial endotoxin. When administered in vivo, LPS induces a cascade of immunoregulatory cytokines which, in turn, act on target cells to mediate the beneficial and deleterious symptoms of the inflammatory response associated with LPS administration and gram-negative infection. Among the first of these cytokines to be associated with LPS-induced manifestations was interleukin-1 (IL-1). IL-1, either alone or in combination with other cytokines, has been demonstrated to induce many of the same physiological responses that are observed after administration of LPS, such as fever, hypoglycemia, induction of other cytokines (as well as classical late acute-phase reactants), resistance to lethal irradiation, increased nonspecific resistance to infection, shock, and death (for a review, see reference 22). These effects may be mediated secondarily by other cytokines or acute-phase proteins induced by LPS or IL-1.

However, the direct demonstration that IL-1 indeed serves as an intermediate in LPS-induced responses can be shown only by blocking the specific LPS-mediated effect with an IL-1 antagonist. For instance, both LPS and recombinant IL-1 (rIL-1) have been shown to induce colony-stimulating factor (CSF) activity in vivo (22). However, this does not prove that IL-1 functions as an intermediate in this LPS-induced phenomenon. Similarly, injection of a sublethal dose of LPS has been shown to mitigate LPS responsiveness 3 days later (referred to as early endotoxin tolerance), and this phenomenon can be simulated by treatment of animals with a combined regimen of rIL-1α and recombinant tumor necrosis factor α (rTNF) (25). However, the finding that these two cytokines synergize to induce a state akin to early endotoxin tolerance does not prove that either cytokine is an intermediate in this LPS-mediated phenomenon.

There are two receptors for IL-1: one which predominates on fibroblasts and T cells (type 1) and one which predominates on B cells and macrophages (type 2) (8, 11). One recently described inhibitor of IL-1 is a 22-kDa IL-1 receptor antagonist (IL-1ra) that occurs naturally as a human macrophage-derived protein with an unglycosylated state and two alternate glycosylated states (2, 3, 11). The cDNA of this protein has now been cloned and the recombinant protein has been produced in an *Escherichia coli* expression system (10). This protein binds to both human IL-1 receptor types (21a) and to the high-affinity (e.g., type 1) murine IL-1 receptor (5, 11) and has been shown to inhibit IL-1-induced prostaglandin E_2 and collagenase secretion from synovial cells in vitro (4). In this study, we have utilized recombinant IL-1ra (rIL-1ra) to test whether (i) IL-1 is an intermediate in the induction of LPS-induced CSF and (ii) IL-1 is an intermediate in the induction of early endotoxin tolerance induced by LPS.

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Five- to 6-week-old C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine) were used in all experiments. To test the efficacy of rIL-1ra to block LPS-induced CSF, groups of four to five mice (per treatment per experiment) were injected intraperitoneally with the indicated combinations of pyrogen-free saline (Abbott Laboratories, North Chicago, Ill.), rIL-1ra (generously supplied by Robert Thompson, Synergen, Inc., Boulder, Colo.; batch 8908), and LPS (25 μg per mouse). Recombinant murine IL-1α (rIL-1α) was generously provided by Peter Lomedico (Hoffmann-LaRoche, Inc., Nutley, N.J.). The LPS used in this study was prepared from *E. coli* K235 by the method of McIntire et al. (17). All reagents were prepared in pyrogen-free saline. Six hours after injection of saline, LPS, or rIL-1ra, mice were bled and the sera were pooled and assayed for CSF activity. CSF activity was measured as the ability of the sera to support the formation of granulocyte-macrophage colonies from murine bone marrow progenitors in semisolid agar, as previously described (12). In preliminary experiments in which rIL-1α was used to induce CSF, mice were bled after 3 h. To assess the efficacy of rIL-1ra to block LPS-induced early endotoxin tolerance, mice were injected on day 0 with saline, LPS, or rIL-1ra, and the mice were challenged 3 days later (day 3) with saline or LPS. Again, the ability to respond to LPS to produce CSF was used as an indicator of LPS responsiveness. Previous studies have shown that administration of a sublethal dose of LPS results in a markedly decreased capacity to respond to a challenge injection of LPS 3 to 4

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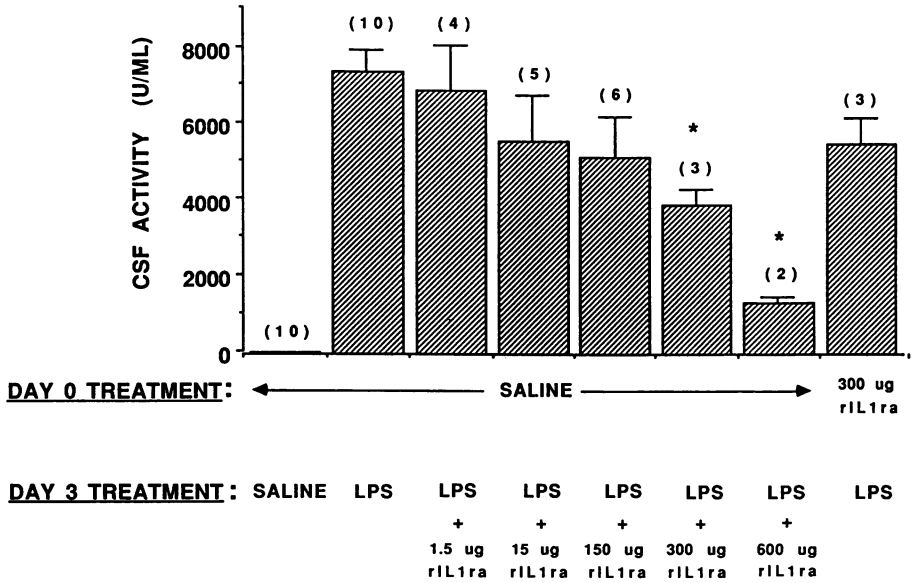

FIG. 1. Effect of rIL-1ra on LPS-induced CSF. Saline or 300 μg of rIL-1ra was injected into C57BL/6J mice (four to five mice per treatment group per experiment) on day 0. Saline or 25 μg of *E. coli* K235 LPS was injected on day 3. Serum samples for CSF determination were obtained 6 h after injection on day 3. Numbers in parentheses indicate the number of separate experiments for each treatment group. Asterisks indicate group means which were found to be significantly different by Student's *t* test ($P < 0.05$) from mice that had received saline on day 0 and LPS on day 3. Six hundred micrograms rIL-1ra induced <20 CFU/ml.

days later to produce CSF (15, 26). Differences between treatment groups were assessed by using an unpaired Student's *t* test.

The effect of rIL-1ra upon LPS-induced CSF activity was first examined (Fig. 1). At the highest dose tested (600 μg), simultaneous administration of rIL-1ra and LPS inhibited LPS-induced CSF by >80% ($P = 0.002$). Lower doses of rIL-1ra blocked production of CSF in a dose-dependent fashion. rIL-1ra exhibited no detectable CSF-inducing capacity of its own, even when as much as 600 μg per mouse was injected (<20 CFU/ml), and in no case was it toxic, even at the highest dose (600 μg per mouse = 33 mg/kg of body weight) tested. Inhibition of LPS-induced CSF by rIL-1ra could be observed even if the antagonist (300 μg of rIL-1ra per mouse) were administered 3 days prior to LPS injection. This carry-over effect of the rIL-1ra (~25% inhibition) is equivalent to the blocking capacity of 15 μg of rIL-1ra administered simultaneously with LPS (Fig. 1). The observed decrease in bone marrow colony number was not accompanied by any apparent decrease in colony size. When added directly to the CSF bone marrow colony assay, rIL-1ra neither stimulated nor inhibited myeloid stem cell proliferation (data not shown).

However, since rIL-1ra and IL-1 compete for occupancy of the same receptor, and yet only a low percentage of receptor occupancy is required for IL-1-induced signal transduction (4), it is possible that even 600 μg of rIL-1ra was not sufficient to compete for all of the IL-1 induced by LPS. The results of a series of preliminary experiments in which we examined the efficacy of rIL-1ra to inhibit rIL-1α-induced CSF would suggest that this is not the case. Previous studies have shown that 300 ng of rIL-1α induces the same level of CSF as 25 μg of LPS, although peak activity is observed earlier than for LPS, at 3 h postinjection (22). Simultaneous administration of 10 or 50 μg of rIL-1ra was found to inhibit peak rIL-1α-induced CSF (11,142 ± 1,150 CFU/ml) by 52 and 80%, respectively. In a second preliminary experiment, a higher dose of rIL-1ra (250 μg) was administered 1 h prior to administration of rIL-1α (300 ng). Peak CSF activity was ablated (8,772 ± 682 to <20 CFU/ml). However, in this same experiment, administration of the antagonist 1 h after rIL-1α resulted in a significant induction of CSF (4,451 ± 605 CFU/ml); nevertheless, the inhibitor was still highly efficacious (26% inhibition) when given substantially after rIL-1α injection.

Treatment of mice with rIL-1ra also partially reversed induction of early endotoxin tolerance by LPS. In the absence of rIL-1ra, exposure to LPS on day 0 results in a markedly diminished capacity to respond to LPS 3 days later to produce CSF (Fig. 2; $P < 0.001$), as previously reported (15, 26). The capacity of rIL-1ra to reverse induction of early endotoxin tolerance was also dose dependent. When 300 μg of rIL-1ra was administered simultaneously with the initial (day 0) LPS injection, CSF production in response to the challenge (day 3) injection of LPS was increased over tolerant-state levels by almost 100% ($P = 0.001$).

Since rTNF has also been shown to induce CSF activity in vivo (22), some portion of the LPS-induced CSF activity may be due to LPS-induced TNF. A previous study demonstrated that administration of anti-TNF antibody to mice failed to reduce LPS-induced CSF by more than ~50% (even when a 10-fold excess of the concentration that led to 50% inhibition was administered). This is indirect evidence that TNF is participatory, but not singular, in the pathway by which LPS induces CSF (23). The finding that rIL-1ra had no effect on LPS-induced TNF production (data not shown) is consistent with the fact that inhibition of LPS-induced CSF by rIL-1ra was incomplete, perhaps due to the contribution of TNF-induced CSF. Alternatively, TNF-induced IL-1 (7, 9) may contribute in part to the induction of CSF by LPS. However, if this were the case, then one would predict that rIL-1ra would completely ablate the LPS-induced CSF re-

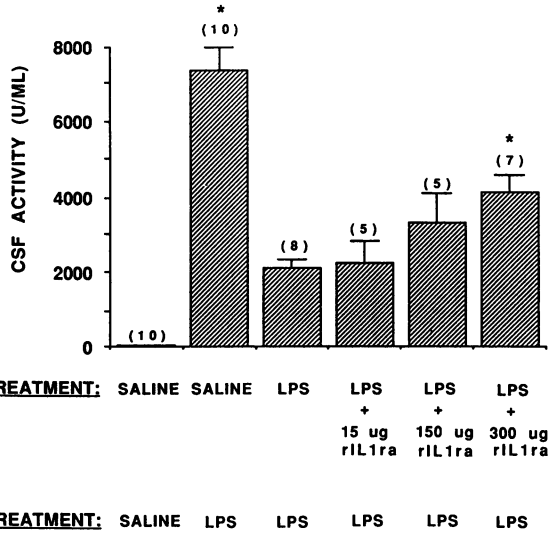

FIG. 2. Effect of rIL-1ra on LPS-induced early endotoxin tolerance. Saline, 25 μg of *E. coli* K235 LPS, or 25 μg of LPS plus the indicated dose of rIL-1ra was injected into C57BL/6J mice (four to five mice per treatment group per experiment) on day 0. One day 3, mice were reinjected with saline or 25 μg of LPS and CSF activity was determined on sera collected 6 h later. Numbers in parentheses indicate the number of separate experiments for each treatment group. Asterisks indicate group means which were found to be significantly different from mice which had received only LPS on days 0 and 3.

sponse. Thus, it is likely that both IL-1 and TNF act as intermediates in induction of CSF by LPS. Other LPS-induced cytokines, such as interferon (IFN), have been shown to antagonize CSF activity in vitro (13), so it is possible that the effect or rIL-1ra could be indirect, by augmentation of IFN levels. When injected alone, up to 600 μg of rIL-1ra failed to induce IFN activity, and 600 μg of rIL-1ra had no inhibitory effect on IFN activity induced by 25 μg of LPS (data not shown).

The findings that (i) rIL-1ra binds preferentially to the murine type 1 IL-1 receptors found predominantly on fibroblasts and T cells, but not to mouse type 2 receptors expressed on macrophages and B cells (5, 11) and (ii) athymic nude mice respond normally to LPS to produce CSF (16) imply that LPS-induced CSF is predominantly of fibroblast, and not of macrophage or T-cell, origin. Indeed, this hypothesis is consistent with previous studies in which it was demonstrated that both IL-1 and TNF stimulate fibroblasts in vitro to produce CSF (18, 27) and that the in vivo production of IL-1 and TNF after administration of LPS is a very early response (6, 11) which precedes the appearance of LPS-induced CSF (12; for a recent review, see reference 24). Nonetheless, the incomplete ablation of LPS-induced CSF by rIL-1ra may be due to triggering of a subpopulation of cells via type 2 receptors.

rIL-1ra does not have any activity in the bone marrow colony assay, indicating that it does not antagonize CSF activity in vitro by binding CSF or by inhibiting the action of IL-1 generated by cells present during the assay. That inhibition of LPS-induced CSF by rIL-1ra is observed, even 3 days following administration of rIL-1ra, suggests that rIL-1ra is neither degraded nor eliminated immediately from circulation. Since rIL-1ra carry-over may inhibit LPS-induced CSF production on day 3, the observed reversal of CSF production during early endotoxin tolerance may actually be even more dramatic than indicated. The finding that rIL-1ra partially reverses the induction of early endotoxin tolerance is consistent with previous work which demonstrated that LPS-induced early endotoxin tolerance could be simulated by injection of both rIL-1α and rTNF; neither alone induced tolerance (25). Thus, rIL-1ra appears to block LPS-induced IL-1 from contributing to the synergistic induction of early endotoxin tolerance. Collectively, the results support the position that IL-1 is indeed an intermediate in the pathway for LPS-induced CSF and illustrates the requirement of IL-1 for the initiation of early endotoxin tolerance by LPS.

Although rIL-1ra has been proposed for use in the treatment of inflammatory diseases which involve aberrant production of IL-1, such as rheumatoid arthritis (1), its application may also be extended to cases of severe systemic gram-negative infection, burn injury, or trauma when initiation of tolerance may hamper the adequate immune response of the individual (14, 19–21).

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